GENETIC REACTIONS IN NICOTIANA. I. COMPATIBILITY

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GOODSPEED (1933) has summarized his conclusions as to the cytogenetic relationships of some 40 species of Nicotianas which he has had under observation at the UNIVERSITY OF CALIFORNIA for a number of years. The American species possess 9, 10, 12, or 24 pairs of chromosomes. [There is no dispute about the chromosome numbers for the various species except a so-called *N. viscosa* listed by CHRISTOFF (1928) as having 24 pairs. I suspect that the form was wrongly identified but can not suggest the correct designation. Though the work of CHRISTOFF was done in this laboratory, I have no notes on these plants and have no seeds from which to grow them]. The Australasian species, all apparently closely related, possess 16, 20, 22, 24, or 32 pairs. After considering both the genetic and the taxonomic evidence, he groups the species as follows. There is an *alata* group, including *N. alata* Link and Otto, *N. Langsdorffii* Weinm., *N. Sanderae* Hort., and *N. bonariensis* Lehm. (n = 9); a *longiflora* group, consisting of *N. longiflora* Cav. and *N. plumbaginifolia* Viv. (n = 10); a *tomentosa* group, including *N. tomentosa* Ruiz and Pavon, *N. tomentosiformis* Goodspeed, *N. glutinosa* L., and *N. wigandioides* Koch and Fint. (n = 12); a *glauca* group, containing *N. glauca* Grah., *N. Raimondii* Macbride, *N. paniculata* L., *N. solanifolia* Walp., *N. cordifolia* Phil., and *N. undulata* Ruiz and Pavon (n = 12); a *Cavanillesii* group, including *N. Cavanillesii* Dun., and *N. pampasana* O. Kuntze (n = 12); an *acuminata* group, containing *N. acuminata* (Grah.) Hook., *N. caudigera* Phil., and *N. attenuata* Torr. (n = 12); and a *repanda* group, consisting of *N. repanda* Willd., *N. Stocktoni* Brandg., and *N. nesophila* Johnston (n = 24). There is also a natural Australasian group as noted above, typified by *N. suaveolens*. Certain, if not all, of the 24-chromosome species are believed to be allopolyploids. For example, there is considerable evidence that *N. Tabacum* derives from hybrids between a member of the *tomentosa* group and *N. sylvestris*; and there is somewhat less convincing evidence that *N. rustica* involves two species of the *glauca* group, of which *N. paniculata* is almost certainly one, and that *N. Bigelovii* S. Wats. involves *N. attenuata* Torr. and possibly *N. trigonophylla* Dun.

Since GOODSPEED and CLAUSEN have concentrated their attention upon this genus for more than twenty years, and since their studies have involved several Australasian and American species not used by other workers, one may accept the above idea of the evolution of the group as the best available. I may add that my own experience, though more casual,

leads me to endorse it in all its essential points. I should not distinguish *N. plumbaginifolia* Viv. from *N. longiflora* Cav. or *N. tomentosiformis* Goodspeed from *N. tomentosa* Ruiz and Pavon as being forms worthy of specific rank, and I should question several other points; but these are minor details. [The stocks which I have had of *N. plumbaginifolia* cross readily with *N. longiflora*, and those of *N. tomentosiformis* (called *N. Rusbyi* in the previous publications from this laboratory) cross readily with *N. tomentosa*. The F_1 plants from both crosses are perfectly fertile].

In general, then, there is an agreement as to the probable evolution of the Nicotiana species, which rests upon as extensive a series of investigations as has been recorded. I do not intend to discuss this problem directly, therefore, either in this paper or in the related papers which will follow. What I propose to do at this time is to present certain data bearing on the reactions of a representative group of these Nicotiana species with each other. This is done as a matter of record preliminary to a discussion of patterns which reacting genoms produce after union in hybrids. These reactions, in turn, form part of the foundation supporting conclusions that I have been driven to make regarding the type of variation which appears to me to play the major rôle in natural evolution.

EXPERIMENTAL WORK

Observations upon the compatibilities of some of the species selected for study have been reported by several of my students, among whom may be mentioned KOSTOFF, CHRISTOFF, BRIEGER, and MCCRAY. I have used their work freely for comparison; but my observations, in nearly every case, have been independent and have been made upon different material. Cytological examinations have also been made upon all the pure species and upon about half of the possible hybrids. Some considerable repetition has thus ensued; but this seemed desirable, since the studies of THOMPSON (1930), WATKINS (1932), and MÜNTZING (1933), on compatibility, have been based, to a considerable extent, upon Nicotiana.

The 12 species groups (table 1) selected for systematic study need a little explanation. Under *Sanderae*, *alata* is included, on the ground that nearly all the *alata* plants in cultivation are merely white-flowered segregates from the *Sanderae* group. The two types, though self-sterile, are perfectly fertile together in the sense that the term is ordinarily used. It is probable that all such segregates react similarly to other species, except that long-flowered plants are not dependable as females on account of the mechanical difficulties involved. Similarly, *longiflora* and *plumbaginifolia* are grouped under *longiflora; tomentosa* and *tomentosiformis* (previously called *Rusbyi* in publications from this laboratory) under *tomentosa;* and *Bigelovii* and *quadrivalvis* under *Bigelovii*. Each pair has been proven to hybridize

TABLE 1

Compatibilities of certain Nicotiana species.

Females	N. San.	N. Lang.	N. long.	N. glauc.	N. glut.	N. pan.	N. tom.	N. syl.	N. suav.	N. Big.	N. rust.	N. Tab.
N. Sanderae 9	—	X HL	X HM	X WE	X P	WS HM	X	X P	X WS	X P	X	X HD
N. Langsdorffii 9	HL	—	X	X HS	X	X	X	X	X P	X	X	X
N. longiflora 10	HM	X	—	WE HS	X	X	X P	X	X	X	X	X WE
N. glauca 12	WE HS	WE HS	WE HS	—	X	X	X HM	X WS	X	X	X	WE HM
N. glutinosa 12	X P	WS	X P	WE HD	—	P WS	WE WS	WE HD	X P	WE HM	X	WE HS
N. paniculata 12	WE HM	WS HD	X WS	X HS	WE HS	—	X	X P	WE WS	X HM	X HM	X HD
N. tomentosa 12	X	P	X	X HM	WE	X	—	X HM	X	WE WS	X	WE HL
N. sylvestris 12	X P	X	X	X	X	X	X HM	—	X	X	X	WE HL
N. suaveolens 16	P WS	X WS	HM	X HS	HM	X P	X	X	—	HM	X	X WS
N. Bigelovii 24	X P	X WE	X WE	X HM	HM	X WE	X HM	X WE	HM	—	X	HM
N. rustica 24	WE HS	WE HM	X WE	WE HS	X WE	HM	X WE	X	X WE	WE HM	—	WE HL
N. Tabacum 24	X HD	WE HS	WE WS	HM	HS	X WE	HL	HL	X WS	X HM	X HL	—

freely and to produce perfectly fertile hybrids. Moreover, each pair is practically identical, taxonomically, except in certain size relations.

To term this group of species "representative," may seem somewhat optimistic. There are no forms from the groups designated by GOODSPEED as *corymbosa*, *Cavanillesii*, *acuminata*, and *repanda*. Nor is the distinctive group, typified by *N. trigonophylla* Dun. and grown here also in a larger-flowered variety received under the name *N. Palmeri* Gray, included. But not all these apparent deficiencies are real, as the following words of explanation will show.

The *corymbosa* group is known to me only from dried material; but I suspect, from its characteristics, that it will prove to have a relatively low compatibility with most of the other species. *Repanda* has been grown but was not used in crosses. This was a real oversight, as it presumably will prove to be compatible with several other groups.

Cavanillesii has not been used by us in a sufficient number of crosses to determine accurately its relationship with other species, but apparently it stands somewhat apart from them. In two combinations, (*suaveolens* ×*glauca*)×*Cavanillesii* and *rustica*×*Cavanillesii*, dwarf seedlings covered with tumorous growths were obtained, the plants persisting for several months without increasing much in size. KOSTOFF believes that this reaction (the tumors) is immunological in its nature. Attempts at crosses on *longiflora*, *rustica*, and *Tabacum* yielded only maternals. Using *Cavanillesii* as female, MCCRAY (1932) failed with *acuminata* and *caudigera* pollen.

N. trigonophylla Dun., *N. caudigera* Phil., and *N. acuminata* (Grah.) Hooker, might have been included in table 1, as they were tested with all the other species. They were omitted because I possessed no wholly satisfactory material of the few hybrids which had been obtained.

N. trigonophylla was crossed reciprocally with all the species listed in table 1, with no result beyond parthenocarpic development, except that reciprocal hybrids with *Bigelovii quad.* and two hybrids of the combination *trigonophylla*×*tomentosa* grew to maturity. In so far as the larger-flowered *trigonophylla* (the so-called *Palmeri*) was tested, it gave no hybrids other than on *tomentosa* and on *tomentosiformis*, except that KOSTOFF obtained one hybrid when pollen was used on the combination *rustica humilis* ×*rustica texana*.

N. trigonophylla is possibly one of the components of *N. Bigelovii*, as GOODSPEED suggests. There is also the possibility that it is connected, in some way, with *nudicaulis*. They resemble each other rather closely; and the hybrid *nudicaulis*×*trigonophylla* is easily obtained, though, in our experience, the reciprocal fails. It is interesting to note the compatibility of *trigonophylla* and the so-called *Palmeri*, North American species, with

the *tomentosa* group, which are South American species with wholly different characteristics.

N. caudigera Phil. was tested reciprocally with all the species of table 1 and with *trigonophylla* and *acuminata*. Reciprocal crosses were obtained in the combinations of *acuminata* and *caudigera*, and the hybrids showed a slight degree of fertility. All other combinations failed; though with *alata*, *Bigelovii*, and *suaveolens* pollen, weak seedlings were obtained which did not flower. It is only fair to say, however, that the number of pollinations made in these attempts at hybridization (3–6) was not sufficient to be certain of the reactions of *caudigera* with other species. It should also be noted that plants of the pure *caudigera* did not grow well under our conditions. The leaves were deciduous, and the plants always died in a month or so after flowering. Possibly these peculiarities affected adversely any hybrids which may have been obtained, though I suspect that, in some degree, they are normal characteristics of the plant.

N. acuminata (Grah.) Hooker was also tested reciprocally with all the plants of table 1, and with *trigonophylla*, *nudicaulis*, and *caudigera*. Besides the hybrids with *caudigera*, already mentioned, no crosses were obtained, though GOODSPEED produced hybrids from the combination *Tabacum*×*acuminata*. I also find among my records that seeds were obtained with pollen of *glauca* and of *tomentosiformis*. Since no further records were made, and I was absent when these seeds were planted, I must assume that they did not germinate.

Let us sum up these extraneous observations independently of the more consistent records found in table 1. The *corymbosa* group appears to have been differentiated rather markedly from most of the other Nicotianas, while the *repanda* group appears to have had less differentiation and will probably be found to exhibit compatibility with at least a few species in our selected group; but since these conclusions are drawn solely from the taxonomic evidence, they are uncertain. The *Cavanillesii* group is another highly differentiated branch of the family from which no mature hybrids were obtained in combination with other species. Dwarf seedlings resulted in two cases which, in their chromosome relationships, were (16×12)×12, and 24×12. The *trigonophylla* group appears to be closely related to *N. Bigelovii* S. Wats. and also to *N. nudicaulis* S. Wats., but the latter hybrid is obtained only when the cross is 24×12. Two other hybrids were *rustica* (24)×*trigonophylla* (12) and *tomentosa* (12) or *tomentosiformis* (12)×*trigonophylla* (12). *Caudigera* and *acuminata* evidently belong together and are not closely related to the other species tested. Such relationship as is shown has little apparent connection with chromosome number. GOODSPEED gets 24×12 (*Tabacum*×*acuminata*). We obtained weak seedlings

in combinations 12×9 (*caudigera*×*alata*), 12×16 (*caudigera*×*suaveolens*), and 12×24 (*caudigera*×*Bigelovii*); while no seedlings were obtained in the reciprocals. More extensive tests would perhaps change the significance of these results.

The compatibilities shown in table 1 are reasonably dependable, since in no case were fewer than 25 pollinations made; but they are not strictly comparable, as in several instances over 1000 pollinations were made.

The table was made up in the following way. Two compartments have been used for each combination. In the first, the usual occurrence is entered; in the second, the less frequent occurrence is shown. Thus X (no stimulation), P (parthenocarpy), WE (weak embryos,—that is, non-germinating seeds having embryos), or WS (weak, non-flowering seedlings), may be entered in compartment one; while HL (large hybrids showing great heterosis), HM (hybrids close to the average size of the parents), HS (small hybrids), or HD (dwarf hybrids) may be entered in compartment two. This system makes it possible to compare the compatibilities of any two species at a glance, provided one exercises a little caution in assigning meanings to the choice of compartments and to the use of the designation X. If a hybrid is assigned to compartment one, it means that roughly two capsules with a fair amount of seed will be obtained out of three pollinations. The use of compartment two, however, means that the hybrid is really difficult to make. The use of the sign X, on the other hand, includes two phenomena. If the pollen tubes reach the ovary, some sort of stimulation usually takes place (see KOSTOFF, 1930), though this is not always the case. Complete failure, therefore, may represent either true incompatibility or deficiency in pollen-tube length.

Fifty-two flowering hybrids were produced. [EAST and HAYES (1912) record hybrids in the cross *Langsdorffii*×*Bigelovii;* yet no herbarium specimen or photograph of the plants was preserved. The only note regarding the matter now available is a record card giving the germination percentage of the seeds and a statement about the vigor of the plants. I suspect that the card itself was made out when the seed was obtained, and that the other entries should have gone on the card of some other hybrid. At all events, numerous attempts to produce such hybrids during succeeding years have failed]. They will be discussed in the second paper of this series. Here I wish to deal only with compatibility.

It has been suggested that if two related species differ in chromosome number, it is easier to obtain a hybrid when the species with the larger chromosome number is used as female (THOMPSON 1930, WATKINS 1932, MÜNTZING 1933). Our data tend to confirm this idea. There are 18 crosses where no reciprocals were obtained. Among these cases there are 5 where the chromosome numbers of the parental species are the same. Of the 13

remaining examples, 11 are cases where the female parent has the higher number. The exceptions both involve *paniculata*. There are 17 instances where reciprocal hybrids were obtained, but the combinations *rustica* with *paniculata* and *Sanderæ* with *Langsdorffii* may be omitted, because it is known that the difficulty of obtaining hybrids when the short-styled plant is used as the male is largely one of insufficient pollen-tube growth. Four others, *longiflora* with *glauca*, *glauca* with *tomentosa*, *tomentosa* with *sylvestris*, and *suaveolens* with *Bigelovii*, show no discernible differences in reciprocal compatibility, although two of these crosses are between parents of unlike chromosome numbers. There are 11 examples where the cross is easier to make one way than it is the other. *Bigelovii*×*Tabacum* and *rustica*×*Tabacum*—both combinations where n=24—are easier to obtain than the reciprocal combinations; but in the remaining 9 combinations the pairs in question differ in chromosome number, and the cross is invariably easier to make where the female has the higher number. It does not appear from table 1 that *paniculata*×*Sanderæ*, *Tabacum*×*Sanderæ*, and *glauca*×*Langsdorffii*, are easier to combine than in the reciprocal manner, but such is the case.

In addition to these facts, I believe that some weight may be attached to the differences in behavior where no mature hybrids were obtained. One may, perhaps, omit the case of *paniculata-Sanderæ*. Mature hybrids were obtained with difficulty in both combinations, but more seeds germinated giving weak seedlings in the combination *Sanderæ*×*paniculata* (9×12) than in the reciprocal. In each case, however, all three types of reaction were observed. There are seven examples where weak seeds, having embryos but not able to germinate, were obtained when the parental species having the higher chromosome number was used as the female, while no stimulation was observed when they were used as the male. In addition, *Tabacum*×*longiflora* yielded weak seedlings, while *longiflora*×*Tabacum* yielded seed with embryos too weak to germinate. The one exception to the rule is *paniculata*×*suaveolens* (12×16). This combination gave weak seedlings, while the reciprocal gave only parthenocarpic seeds.

DISCUSSION

Probably no single explanation will suffice for all these facts and for similar facts that have been adduced in other genera. In order to simplify matters, therefore, it seems desirable to omit all cases where it is known that pollen tubes of a short-styled species do not reach the ovary of a long-styled species. Here the difficulty in question is one having to do either with a fairly definite ultimate length of pollen tube or of a slower growth-rate. At all events, when opportunity for fertilization is presented, hybridization frequently takes place—as, for example, in *Sanderæ*×*Langsdorffii*

and in *paniculata*×*rustica*. Nevertheless it is also possible that true differences in compatibility are present in addition to the pollen-tube difficulties. This may be the case in combinations of *rustica* and *paniculata*. Other physiological difficulties connected with pollen-tube growth are also known. The bursting of tetraploid Datura pollen tubes in diploid tissue is an example.

Omitting the above cases without prejudice as to whether the ultimate cause may not often be the same in both groups, there are three explanations of the rather generally greater compatibility of species unions when the species having the higher chromosome number is used as the mother. [KIHARA and NISHIYAMA (1932) have suggested that differential development of hybrid seeds in reciprocal crosses is due to different potencies of the male nuclei in activating the egg and the fusion nucleus. If the chromosome number of the male gamete is the larger, rapid growth of endosperm and embryo are said to result; if it is smaller, the reverse is true. I have not been able to convince myself that the facts accord with this view. It is generally believed, and with considerable reason, that the female, through the cytoplasm of the egg, controls all early divisions in the filial generation. Some time is necessary before the filial genes become active in directing development]. They are: (a) cytoplasmic differences, (b) disturbed endosperm relations, and (c) specific immunity reactions.

These interpretations, the first two of which have been considered minutely by THOMPSON, WATKINS, and MÜNTZING, and the last by KOSTOFF, have been thought to be quite independent of one another. It is true that they could turn out to be different and independent of one another had we an exact knowledge in physiological terms of what goes on at fertilization and during early embryology. As they stand, however, they are mere statements in vague terms that there are broader possibilities for harmonious chemical reactions during early development when the embryo sac machinery is controlled by the parent with the larger number of chromosomes than there are when it is controlled by the parent with the smaller number of chromosomes. The only difference between them concerns the type, or possibly the location, of the chemical action. The only matter warranting discussion, then, is whether there are any facts which delimit the problem somewhat more precisely.

When KOSTOFF (1929, 1930) suggested that certain inhibition phenomena manifested during the early development of embryo and endosperm in hybrids resembled those interpreted as antigen-antibody reactions by immunologists, it was not well received. It was thought to be a speculation having no basis of fact, because, it was said, the proteins necessary for the production of such reactions can not pass through living membranes. During the past four or five years such criticisms have become untenable.

Detailed studies of reactions in pregnancy and of allergic manifestations have shown that small quantities of proteins sufficient to cause marked reactions can pass living animal membranes. Further, a large and growing literature tends to show that the situation in plants is similar (see complete review by CHESTER 1933). Thus one must conclude that there is an opportunity for maternal tissue to react harmoniously or inharmoniously on developing endosperm and embryo.

Now, since it is an immunological tenet that various kinds of proteins may be produced and enter into specific reactions within a given reaction system (blood stream or cell), it is suggested that there is ordinarily (in cases where extreme segregant gametes are formed, this statement would not be true) a similar distribution of probabilities for harmonious or for inharmonious reactions, whether one considers fertilization and the subsequent changes of early development as matters wholly dependent upon the reactions between embryo sac constituents and male nuclei, or whether one admits the possibility of immunological reactions between maternal and filial tissues.

In allopolyploidy, if A and B are genoms, and x and y represent gene changes, then the polyploid is A+x+B, while the simple constituent is A+y. The mutations x and y must be harmonious with A if the organism is to persist; but x may not be harmonious with y. It happens frequently, therefore, that species A+x and A+y, derived from the same stock, will not cross. By similar argument, A+x+B and A+y may be incompatible reciprocally. But there is the possibility of a broader series of cytoplasmic products being initiated under the control of A+x+B than there is under the control of A+y. Thus it seems reasonable to assume greater likelihood of harmonious development of a hybrid when the mother is a pure species of some type of allopolyploid origin than if the mother is one of the constituents. And the same argument holds in case one admits the possibility of protein reactions similar to those designated as antigen-antibody reactions.

A further development of this idea also has some plausibility. It is known that when hybrids show a border-line fertility, the eggs are more likely to be functional than the sperm. In animals, there is an enormous amount of egg cytoplasm manufactured under the influence of the entire maternal complement of chromosomes, while there is practically no sperm cytoplasm. In plants, the disproportion in the amounts of cytoplasm available in the embryo sac and in the male gametes is less. I suggest that the third cell-division in the embryo sac is an activity associated with the provision of adequate materials of proper type and quantity for the filial generation.

Information is not plentiful on the reactions of autopolyploids with con-

stituent diploids, but that which is available also indicates that the cross is easier to make when the form with the higher chromosome number is used as the mother. Where this difference is not one of pollen-tube growth, and hence possibly a distinct problem, may it not be that greater quantity of cytoplasmic products rather than a larger number of types is the explanation?

It has been emphasized, particularly by MÜNTZING, that disturbed seed development in hybrids of the type we are discussing is due to a change in the relationship between maternal tissue, endosperm tissue, and embryo tissue which normally have the chromosome relationships 2n:3n:2n. How does this view fit in with the idea that breadth of chemical possibilities is the chief causal agent in determining differences in reciprocal compatibilities? An exact answer can be given only when we are certain of the facts in each particular case. MÜNTZING, and also WATKINS, assume 3N endosperm development in these hybrids without proof. Such assumptions are possibly at variance with the facts, for KOSTOFF (1930), working here with Nicotiana hybrids, found N and 2N endosperms as well as 3N endosperms. But assuming that 3N endosperms develop, it still seems reasonable to suppose that the excess chromosomes coming from the female parent will furnish more harmonious environment for the young embryo and more possibilities of nutriment in the germinating seeds if the species with the higher number of chromosomes is the mother.

SUMMARY

An extensive survey of the compatibility between species in the genus Nicotiana corroborates the idea that, in general, greater compatibility is shown when species having higher chromosome numbers are used as females.

It is suggested that conflicting views as to the probable explanation of this phenomenon can be harmonized if one assumes that where two species of a given genus have different chromosome numbers, some type of allopolyploidy may be presumed, and that the parent with the higher chromosome number presents broader possibilities for harmonious chemical reactions during early development if the embryo sac machinery is controlled by the parent having the larger chromosome number.

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GENETIC REACTIONS IN NICOTIANA. II. PHENOTYPIC REACTION PATTERNS

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In a paper entitled "Genetic Reactions in Nicotiana. I. Compatibility," the hybrid compatibility of twelve species groups of Nicotiana was reported. Reasonably adequate tests were made on all the possible combinations. Complete, but less extensive, tests were described on three other species. Of the 132 unions possible in the first group, 52 hybrids were obtained which grew to maturity. In the other tests, 4 mature hybrids were produced, in addition to *nudicaulis*×*trigonophylla*. If the two tests are added, the result is 56 flowering hybrids out of 210 possible unions.

In this paper, I shall describe the phenotypic reaction patterns of these hybrids. (So far as I know, this apt term was first used by SINNOTT in his work on the shapes of cucurbits.) My object is not simply to describe the various hybrids, but rather to inquire as to how a given species reacts with various other species in regard to any stated characteristic. This is a method of study, thus far neglected, that ought to give us genetic information of considerable value.

As a necessary preliminary to these discussions, a few words should be said about the stocks used for the crosses and about the inheritance of certain obvious character differences in varietal crosses.

As GOODSPEED (1933) has stated, most of the Nicotiana species give evidence of polymorphism. *N. sylvestris* and *N. paniculata* are exceptions. Our own stocks, however, had all been inbred until they had reached a high degree of uniformity before they were used in the crossing experiments, aside from *N. Sanderæ*, which is self-sterile. Several genotypes were used as foundation stocks, however, in the species *N. Sanderæ*, *N. Langsdorffii*, *N. longiflora*, *N. glauca*, *N. glutinosa*, *N. paniculata*, *N. trigonophylla*, *N. Bigelovii*, *N. rustica*, and *N. Tabacum*. I think it may be assumed, therefore, that the species involved in the crosses, always excepting *N. Sanderæ*, are as nearly homozygous as one can expect in such experiments. Naturally, in an investigation extending over a quarter of a century, it is impossible to control matters in an ideal way. But several of our reciprocals were made with identical plants. In other instances, a given plant was crossed with several other species.

Much of the Nicotiana polymorphism is exhibited as size differences. One is not far wrong in saying that *N. Sanderæ* and *N. Tabacum* are the only species where numerous qualitative variations are found. In *N. Sanderæ* these qualitative differences are confined, in general, to genes affecting

corolla color. Some idea of the genetic behavior of the anthocyan-producing genes of *N. Sanderæ* may be found by referring to papers cited by EAST (1928); but much remains to be done. *N. Tabacum* varieties vary especially in flower color and in leaf shape. Tobacco flowers are either red or white. The reds are of several kinds, and are due to the influence of two main genes and at least two modifying genes, as reported by SETCHELL, GOODSPEED and CLAUSEN (1922). Our own results from numerous varietal crosses are essentially the same. There is also a set of genes controlling leaf shape, petioled types being partially dominant. Three such genes are described in the paper cited above as having the major influence in determining the differences between petioled and sessile forms. There is little doubt that three gene pairs are the main determiners of naked petiole, fringed petiole, sessile, and auricled leaves; but we feel, from our own experience, that there is a very large series of gene differences which have various modifying effects.

EXPERIMENTAL WORK

General plant reactions

It is not easy to generalize on the way these species react with each other when considering characteristics which may be grouped under the somewhat unsatisfactory phrase *habit of growth*.

All species of Nicotiana form a rather definite rosette from which an erect stem emerges. But the *longiflora* group has a rosette that is markedly flattened, and the *Sanderæ* group and *Langsdorffii* have one that is noticeably flattened; while the remaining species exhibit more spacing between the early leaves. Where *longiflora*, *Sanderæ*, and *Langsdorffii* are brought together (crosses 1, 2, 5, 7), the hybrids invariably show the flat rosette tendency. Where the genoms of these three types are united with the genoms of other species, however, the reaction is a variable one which is not closely correlated with the number of chromosomes contributed by the two parents. *Longiflora* with *suaveolens*, for example, produces more of a characteristic flat rosette than *longiflora* with *glauca*. Again, *Sanderæ* and *Langsdorffii*, when combined with *Tabacum*, give flatter rosettes than when combined with some of the 12-chromosome species.

Similar remarks cannot be made regarding stems. The simple ascending stem which appears after a month or so in the Nicotianas rapidly becomes diffuse to a greater or less degree in *Sanderæ*, *Langsdorffii*, *paniculata*, and *suaveolens*. In other species the stem remains single, though the development of lateral shoots may give the appearance of diffuseness in such species as *glutinosa*, *Bigelovii*, and *rustica*. The diffuseness of *suaveolens* is apparent in all combinations, although there is a reasonable degree of variability in the different types of hybrids. With this exception, the

single erect stem is, as one may say, dominant in combinations where *glauca*, *tomentosa*, or *Tabacum*, is one of the parents.

Of the species being considered here, the only one that is truly arborescent is *glauca;* but all the species are suffrutescent to a greater or less degree. It is interesting to note that hybrid combinations made with *glauca*, of which there are fourteen, all show a definite and individually characteristic reaction pattern which can more easily be referred to the type of the other parent than to its chromosome number. Thus, *tomentosa*, which is more woody and tree-like than *Tabacum*, yields hybrids with *glauca* which are more tree-like than those with *Tabacum;* while the hybrids with *Sanderæ* and *Langsdorffii* have but a slight tendency to become woody and are too stunted to exhibit any tendency to arborescence if they possess it.

The young stems of all hybrids where *glauca* is one parent are hairy, though there is a difference both in the size and the frequency of the hairs in different combinations. *Longiflora*×*glauca* even shows the prickles characteristic of *longiflora*, though they are small and somewhat sparse. In every hybrid, however, the hairs have a tendency to fall off the stems as the stems become older. This trend toward a bald condition begins at different ages in the different hybrids. The stems rarely become truly smooth, and new stem growths are always hairy. The bloom of *glauca* does not appear in hybrids.

Color reactions

Purple anthocyan appears in the stems of certain populations of *Sanderæ*. It is due to a gene *C* which is linked with the self-sterility gene *S*. *Langsdorffii* plants apparently are always *c*. Hybrids between the two species are purple or non-purple, depending on whether *C* has entered from *Sanderæ*. Certain other species, such as *longiflora* and *glauca*, usually (perhaps always) have purple anthocyan spots either at the base of the stem or at the leaf axils. *Tomentosa* and *Tabacum* varieties may have such spots. I have never seen this color, however, in *glutinosa*, *paniculata*, *sylvestris*, *Bigelovii*, or *rustica*. I have followed the transmission of color with particular care in the *glauca* hybrids. There the purple color from *glauca* appears in all hybrids except the *glauca-Langsdorffii* and the *glauca-glutinosa* combinations. In the *glauca-Tabacum* combinations, the color not only appears, but the amount appearing is much greater than that found in *glauca*, even when the variety of *Tabacum* used shows no trace of stem color. Instead of the color being confined to the leaf axils, as in *glauca*, the petioles and veins of the leaves are a bright purple, which, however, gradually fades.

One must suppose, therefore, that *glauca* and most other species show

an antagonism to the full development of stem anthocyan, which is increased by *glutinosa* and *Langsdorffii* genoms to the point of full inhibition, and that these antagonistic genes are absent in at least some of the varieties of *Tabacum*. Since *tomentosa* hybrids made with *glauca* develop the spots of color, the release to full development should come from *sylvestris* genes (if *sylvestris* is a component of *Tabacum*).

In other instances anthocyan appears to be transmitted as if there were no or very little antagonism to its development. White-flowered *Sanderæ* plants transmit no color when combined with species having no anthocyan. But red-flowered or purple-flowered *Sanderæ* reproduce their colors, albeit as if somewhat diluted, in combination with non-anthocyan species. The same may be said of the various reds in *Tabacum*. GOODSPEED and CLAUSEN (1922) found that in crosses of five different *Tabacums* with *sylvestris*, the particular color type of *Tabacum* was transmitted, though there was perhaps a smaller quantity of the coloring material. We have corroborated their results with four different *Tabacum* varieties. When a white-flowered *Tabacum* was used, no flower color appeared in the hybrid.

In many of the white-flowered *Sanderæs* (the so-called *alatas*) and in the *longiflora* group, a slight tinge of purple appears on the back of the corolla. This is never extended in hybrids, though it is present.

The experiences given above hold for all anthocyan-colored flowers, including those of *tomentosa* and *glutinosa*, where dull reds appear, when combinations are made with white-flowered types such as *Sanderæ-alata*, *longiflora*, *sylvestris*, *suaveolens*, and *Bigelovii*. They also hold when species with greenish-yellow flowers (the flowers of *tomentosa* and *glutinosa*, and the outer veins of the flowers of *Bigelovii* develop a little chlorophyll) are used, such as *Langsdorffii*, *glauca*, *paniculata*, and *rustica*, but with some modification.

When *Tabacum* is involved, its red anthocyan is transmitted with very little dilution, no matter which other species is used. When the anthocyan-producing genes enter a cross from species having 9 or 12 chromosomes, however, there is much variation in the way they act. *Sanderæ-Langsdorffii* hybrids, where both species have 9 chromosomes, behave as in varietal crosses except for the addition of chlorophyll. The color follows *Sanderæ*. *Paniculata*×*Sanderæ* behaves similarly, but here the anthocyans are dulled, even though there is less green in the hybrid flowers than when *Langsdorffii* is used. The flowers are larger than in *Sanderæ-Langsdorffii* and appear as if a given quantity of anthocyan and of chlorophyll were spread out over a larger surface than in the parents. If white *Sanderæs* are used, the hybrid flowers are white, with no more green on the exterior of the tube than is often present in the pure (=*alata*) type. In combination with *glauca*, which has a yellow flavone color in addition to the green

chlorophyll, the reds of *tomentosa* and *glutinosa* are modified in quantity, type, and distribution more than are those of *Sanderæ*. *Glutinosa*×*glauca* has slight flakes of red color, while *glauca*×*tomentosa* has only the slightest suggestion of color.

The brighter flavone yellow of *glauca* appears in crosses with *paniculata*, but is not obvious in crosses with *Langsdorffii*. Other combinations of green types with green types have their own characteristics and usually show the influence of both parents.

One gets the idea, therefore, that anthocyans are due to a very limited number of genes whose normal activity is not significantly distorted by the genes of other species, but that chlorophyll production in the greenish-yellow flowered species is highly complex and can be modified in numerous ways, though not prevented, by other gene complexes.

Flower reaction patterns

In table 1 a list of the fifty-two hybrids which attained maturity in the primary investigation is given. For forty-six of the hybrids, certain flower measurements are given which permit comparisons. The length of the pistil is recorded where the pistil is longer than the corolla in one of the parents. The length of the flower, as listed, is taken from the lower end to the constriction of the throat just below the corolla limb. The breadth is the greatest breadth across the corolla lobes. The fourth and fifth measurements are the length of the largest corolla lobe and the length of the adjacent sinus.

These measurements have a certain importance in that they are the only quantitative characters thus far noted which are, for all practical purposes, unaffected by environment (see EAST 1916). Naturally there are variations in flower size on any given plant, but the modal condition always shows a sharp peak. And the frequency distributions of a pure line of plants, when each plant is represented by modal measurements for that plant, are almost identical when the population is grown in small pots where the plant food is restricted and when it is grown under the best of field conditions.

The first point to which I wish to draw attention is that reciprocal crosses are as nearly identical as are the plants of a given parental population. There are seventeen pairs of reciprocals, having the chromosome numbers shown.

Sanderæ×*Langsdorffii*	9 and 9
Sanderæ×*longiflora*	9 and 10
Sanderæ×*paniculata*	9 and 12
Sanderæ×*Tabacum*	9 and 24

Langsdorffii×*glauca*	9 and 12
longiflora×*glauca*	10 and 12
glauca×*tomentosa*	12 and 12
glauca×*Tabacum*	12 and 24
glutinosa×*Bigelovii*	12 and 24
glutinosa×*Tabacum*	12 and 24
paniculata×*rustica*	12 and 24
tomentosa×*sylvestris*	12 and 12
tomentosa×*Tabacum*	12 and 24
sylvestris×*Tabacum*	12 and 24
suaveolens×*Bigelovii*	16 and 24
Bigelovii×*Tabacum*	24 and 24
rustica×*Tabacum*	24 and 24

Measurements were recorded for only one cross in the combinations *glauca-tomentosa* and *tomentosa-sylvestris*, but my notes show that the reciprocals were so similar that they could not be told apart. If, then, these two pairs are included in the total, there are thirteen pairs where the hybrids are identical, for all practical purposes. Seven pairs were made in which the parents used were the same plants, although the measurements of the parental flowers do not check in every case because they were sometimes taken at different flowering periods. In most of the other instances sister plants were used. The four apparent exceptions are not true exceptions. In general characteristics the reciprocals considered as units were similar to each other, but the flower measurements are different because different varieties were used. The *Sanderæ* genotypes were not at all alike in the combination *Sanderæ-Langsdorffii*, though they were similar in the combinations *Sanderæ-paniculata* and *Sanderæ-longiflora*. The cross *tomentosa*×*Tabacum* was made with *tomentosiformis*, while the reciprocal, so-called, was made with *tomentosa*. Two different varieties of *Tabacum* were used in the *Bigelovii-Tabacum* unions, and two different varieties of *rustica* were used in the *rustica-Tabacum* unions.

There are also two identical reciprocals that are not included in table 1 (see Genetic Reactions in Nicotiana. I.). They are the *caudigera-acuminata* combinations and the *Bigelovii-trigonophylla* combinations. One of the latter is represented in figure 3. Thus there are fifteen reciprocals that are comparable genetically, and each member of every pair is identical with the other.

In view of the general interest in reciprocal crosses because of possible plasmatic influence, it is necessary to emphasize, at this point, that in characteristics other than the flower measurements, these reciprocals are also identical when comparable. In other words, reciprocal crosses in

TABLE 1

Flower measurements in various hybrid combinations (millimeters).

HYBRID COMB.	MATERNAL PARENT					HYBRID					PATERNAL PARENT				
	L. PIS.	L. COR.	BR. COR.	MAX. LOBE	ADJ. SINUS	L. PIS.	L. COR.	BR. COR.	MAX. LOBE	ADJ. SINUS	L. PIS.	L. COR.	BR. COR.	MAX. LOBE	ADJ. SINUS
1. *Sand.*×*Langs.*		92	51	28	11		36	27	13	9		18	14	5	5
2. *Sand*×*long.*		42	45	21	10		54	37	19	5		90	50	23	7
3. *Sand.*×*pan.*		100	62	29	9		55	34	16	10		30	10	3	3
4. *Sand.*×*Tab.*		64	40	19	7		39	26	14	8		37	21	9	5
5. *Langs.*×*Sand.*		20	15	5	5		44	34	16	10		84	60	32	13
6. *Langs.*×*glauca*		19	14	6	5		28	15	6	6		30	12	5	5
7. *long.*×*Sand.*		90	52	23	7		53	37	19	5		44	45	21	11
8. *long.*×*glauca*		41	21	10	6		36	20	7.5	3.5		30	12	5	5
9. *glauca*×*Sand.*															
10. *glauca*×*Langs.*		30	12	4	4		28	14	5	5		19	14	6	5
11. *glauca*×*long.*		30	12	4	4		36	20	8	4		41	20	10	6
12. *glauca*×*toment.*	31	30	12	4	4	46	39	22	7	4	48	29	31	12	4
13. *glauca*×*Tab.*		28	10	4	4		43	24	9	4		43	24	10	5
14. *glut.*×*glauca*		26	—	—	—		26	12	6	5		29	12	4	4
15. *glut.*×*sylves.*		25	—	—	—	51	39	33	14	8		88	30	15	9
16. *glut.*×*Big.*		26	—	—	—		26	28	12	6		25	27	12	6
17. *glut.*×*Tab.*		25	—	—	—	40	35	29	12	6		43	31	12	6
18. *pan.*×*Sand.*		29	9	3	3		52	32	14	8		95	60	27	7
19. *pan.*×*Langs.*		28	8	3	3		26	18	7	6		20	14	4	4
20. *pan.*×*glauca*		28	8	3	3		29	11	4.5	4.5		29	10	4	4
21. *pan.*×*glut.*		28	8	3	3		27	14	6	4		26	—	—	—
22. *pan.*×*Big.*		30	9	3	3		37	27	17	14		46	55	27	16
23. *pan.*×*rust.*		30	10	3	3		20	15	5	4		15	15	5	4
24. *pan.*×*Tab.*		30	9	3	3		35	21	8	7		41	23	11	6
25. *toment.*×*glauca*															
26. *toment.*×*sylves.*															

TABLE 1 (*Continued*)

Flower measurements in various hybrid combinations (millimeters).

HYBRID COMB.	MATERNAL PARENT					HYBRID					PATERNAL PARENT				
	L. PIS.	L. COR.	BR. COR.	MAX. LOBE	ADJ. SINUS	L. PIS.	L. COR.	BR. COR.	MAX. LOBE	ADJ. SINUS	L. PIS.	L. COR.	BR. COR.	MAX. LOBE	ADJ. SINUS
27. *toment.*×*Tab.*	36	24	18	6	4	49	33	26	11	7	43	42	24	10	5
28. *sylves.*×*toment.*		88	30	15	9	64	60	21	15	9	36	25	19	7	4
29. *sylves.*×*Tab.*		92	31	16	10		62	33	16	6		53	29	13	5
30. *suav.*×*long.*		43	33	16	12		70	38	18	7		91	53	24	8
31. *suav.*×*glauca*		42	32	15	11		32	19	8	7		30	12	4	4
32. *suav.*×*glut.*		43	33	16	12		43	35	17	13		26	—	—	—
33. *suav.*×*Big.*		42	32	15	11		40	41	20	13		20	26	11	7
34. *Big.*×*glauca*		24	23	11	6		24	14	6	5		28	10	3.5	3.5
35. *Big.*×*glut.*		25	28	12	6		26	29	13	6		26	—	—	—
36. *Big.*×*toment.*	26	25	25	12	6	32	26	25	12	6	36	25	19	7	4
37. *Big.*×*suav.*		20	26	11	7		41	42	21	13		42	32	15	10
38. *Big.*×*Tab.*		25	28	12	6		35	24	12	6		40	22	10	5
39. *rust.*×*Sand.*															
40. *rust.*×*Langs.*		16	15	5	4		18	16	5	4		19	14	6	5
41. *rust.*×*glauca*															
42. *rust.*×*pan.*		15	15	5	4		20	16	5	4		30	10	3	3
43. *rust.*×*Big.*		15	14	4.5	4		25	23	10	8		25	24	12	7
44. *rust.*×*Tab.*		15	15	5	4		30	26	9	8		50	29	12	4
45. *Tab.*×*Sand.*		36	20	9	4		40	27	14	7		64	40	19	7
46. *Tab.*×*Langs.*															
47. *Tab.*×*glauca*		43	24	10	5		42	24	9	4		28	10	4	4
48. *Tab.*×*glut.*		42	30	12	6	41	36	30	13	7		25	—	—	—
49. *Tab.*×*toment.*	44	42	24	10	5	60	40	31	13	8	48	28	28	9	7
50. *Tab.*×*sylves.*		51	28	12	4		61	31	15	5		90	30	16	10
51. *Tab.*×*Big.*															
52. *Tab.*×*rust.*		46	29	11	4		33	24	9	7		19	19	7	5

Nicotiana, no matter what the chromosome numbers happen to be, show no significant differences. In figure 8, two flowers are shown representing reciprocals between *glauca* and *longiflora* (*plumbaginifolia*).

The flower measurements are spread on the record, with the hope that they may be useful, but I am not at all confident that statistical data of this kind will yield many acceptable generalizations. It seems to me that the situation here is similar to that with which geneticists were confronted at the beginning of this century, when the application of methods of gross analysis merely resulted in restricting progress.

The development of the flower can presumably be expressed by some type of exponential curve. One surmises, however, that the growth-curve of the flower is somewhat different from most growth-curves in that there is a marked activity at the opening of the flower, followed by a sudden cessation, rather than a gradually diminishing growth force. What we have here is a set of final measurements of total growth obtained when genoms A+A, A+B, and B+B are involved.

Now when we examine the situation presented by the measurements listed in the table, certain matters are obvious; and they are obvious no matter how the figures are treated, whether arithmetically or logarithmically, whether as straight linear measurements or as combination indices. We see a developmental pattern always influenced in some respect by both parents. Sometimes the influence of one parent is hardly discernible in length of corolla or in spread of corolla or in the amount of lobing; but there is a harmonious development of the organ group as a whole, in which the activity of the genes of each parent can be traced. There is no heterosis (unless one so interprets the *Bigelovii* hybrids). I should interpret this fact as an indication, first, that hybridity is not accompanied here by some mysterious growth force inexplicable by gene activity, as has been suggested by some writers in an endeavor to explain heterosis; and, second, that there is seldom, if ever, a dominant action of genes in the production of a quantitative growth-pattern. Gene actions are additive in the sense that each would seem to have its effect on the final outcome of any particular part of the pattern; but they are not additive in the sense that two genes *A* have twice the effect of a single gene *A*.

When one compares the various crosses with *Tabacum*, it is clear that the twenty-four chromosomes of *Tabacum* sometimes appear to have an overwhelming effect on the end result when pitted against the effect of a smaller number of chromosomes, but this is, I think, illusory. And there are numerous examples where there is no such apparent effect. Consider, for example, the *Sanderæ-Tabacum* union, where the nine chromosomes of *Sanderæ* have such a distinct and positive effect. The same thing may be said, in general, when one takes any given species and compares the various

hybrids obtained with other species. No chromosome generalization holds. The pattern is the thing.

There is also a second point upon which there is substantial evidence.

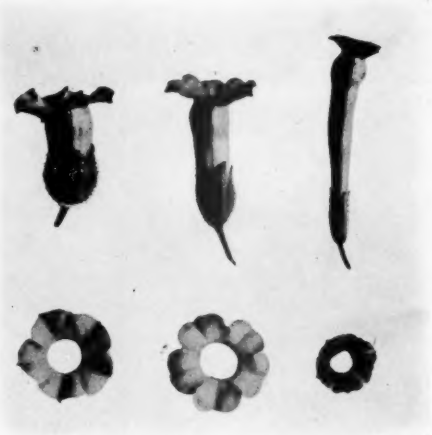

FIGURE 1.—*N. rustica humilis; N. rustica humilis*×*N. paniculata; N. paniculata.* ×1.

In work with qualitative characters one often finds gene combinations that are epistatic to others, gene combinations that shut out the effects of other genes, genes that are suppressors, and genes which, conversely, have a

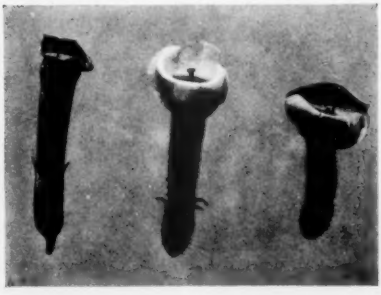

FIGURE 2.—*N. glauca; N. glauca*×*N. Langsdorffii; N. Langsdorffii.* ×1.

releasing effect on the activity of other genes. Little of this is in evidence in the behavior of the gene combinations which produce the flower patterns. The reactions are harmonious. There is only one exception in the table. The variety of *Bigelovii* used most frequently in crosses is the small-

flowered variety, *quadrivalvis*. The typical *Bigelovii*, however, has very much larger flowers (see figures 3 and 4). In crosses with *Bigelovii quadrivalvis* the hybrids obtained had larger flowers than one would expect, on the basis of what was found in hybrids between other species. It seems,

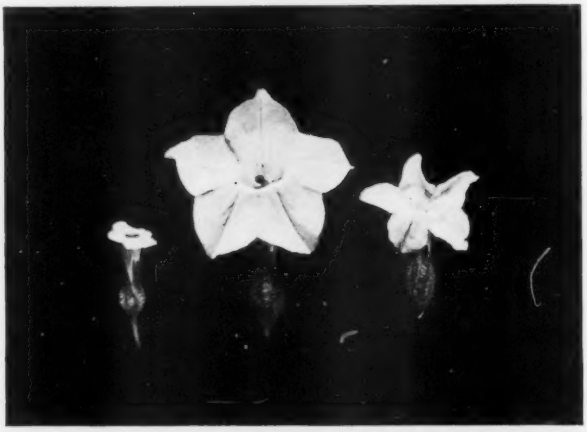

FIGURE 3.—*N. trigonophylla; N. trigonophylla*×*N. Bigelovii quad., N. Bigelovii quad.* ×3/4.

FIGURE 4.—*N. suaveolens; N. suaveolens*×*N. Bigelovii quad.; N. Bigelovii quad.; N. rustica texana*×*N. Bigelovii quad.; N. rustica texana.* ×3/4.

therefore, as if *Bigelovii quadrivalvis* has a tendency for larger flowers that is suppressed in the pure variety but is released in species hybrids.

The exact reaction pattern that results in the particular type of hybrid flower obtained is more clearly seen in the photographs. Visualization of

the flowers from the measurements is much more difficult, since one can

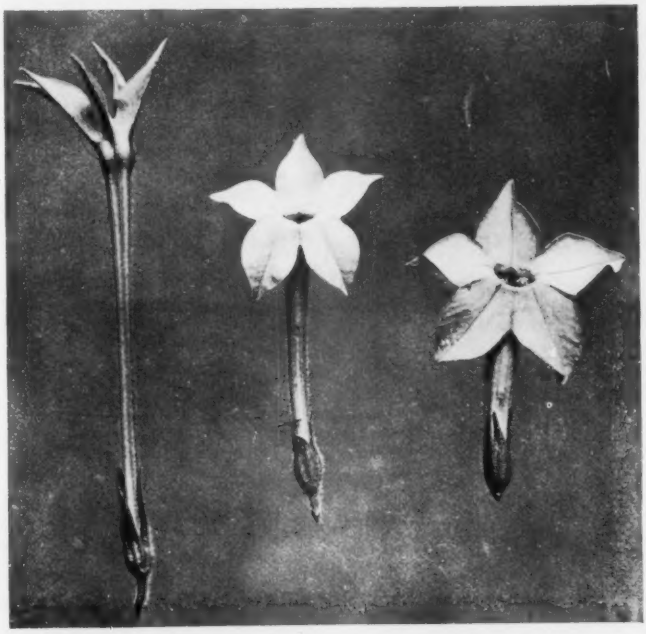

FIGURE 5.—*N. longiflora; N. longiflora*×*N. Sanderæ; N. Sanderæ.* ×3/4.

FIGURE 6.—*N. Bigelovii quad.; N. Bigelovii quad.*×*N. Tabacum Havana; N. Tabacum Havana; N. Tabacum Havana*×*N. glutinosa; N. glutinosa.* ×3/4.

not get a clear idea of the width of the tube and has no data from which to reconstruct the shape of the tube.

Let us consider, first, the hybrids between the species which produce

the yellow-green flowers. These flowers are tubular without much spread to the limb. The three primary species which we have used are: *N. Langsdorffii* (9) of the *Sanderæ* group, and *N. glauca* (12) and *N. paniculata* (12), of the *glauca* group. The *paniculata*×*glauca* hybrid is, as one might expect, of an intermediate character, for the flowers of the parents are similar.

FIGURE 7.—*N. paniculata; N. Sanderæ alata*×*N. paniculata; N. Sanderæ alata; N. Langsdorffii*×*N. Sanderæ alata; N. Langsdorffii.* ×3/4.

The size is approximately that of the *glauca* parent, the spread of the corolla being even slightly greater. The *glauca* (12)×*Langsdorffii* (9) hybrid (figure 2) is also intermediate with slightly more influence of *glauca* seen in the size and shape of the tube and in the characteristics of the calyx; but with the *Langsdorffii* influence obvious in the shape and size of the limb. The *rustica* (24)×*paniculata* (12) hybrid is shown in figure 1. *Paniculata* is presumably one of the components of *rustica;* and, owing possibly to the effect of the twenty-four other chromosomes, the hybrid

appears to stand somewhat closer to *rustica*. It is not certain, however, that this admission is in accordance with the facts, though a marked influence of *rustica* in this case would not invalidate our position any more than the marked influence of *alata* (9-chromosome) in crosses with species having higher chromosome numbers would demonstrate it. At all events, when the influence of the two parents of this hybrid is analyzed, length

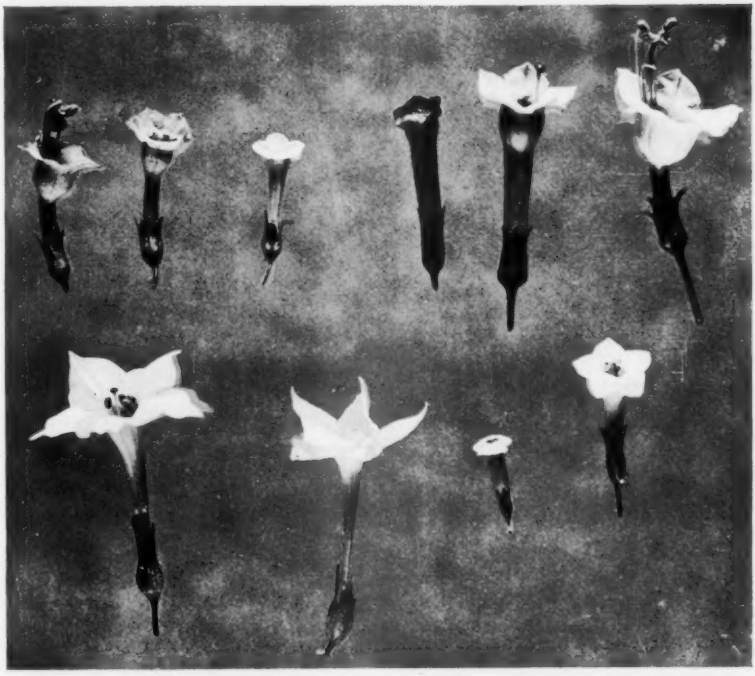

FIGURE 8.—Upper row:: *N. tomentosiformis; N. trigonophylla (Palmeri)* ×*N. tomentosiformis; N. trigonophylla (Palmeri); N. glauca; N. tomentosa*×*N. glauca; N. tomentosa.* Lower row: *N. tomentosiformis*×*N. Bigelovii quad.; N. Bigelovii quad.; N. nudicaulis; N. tomentosa*×*N. nudicaulis.* ×3/4

and breadth taken on a logarithmic basis and various minor effects considered, it is not too certain where the hybrid stands. Moreover, precise analysis of the situation would require a knowledge of the second constituent of *rustica*. Of it we know nothing, though it may be assumed, I believe, that the second component has a corolla limb that is more lobed than *paniculata*. No lobing is apparent in the *glauca-paniculata* or *glauca-Langsdorffii* unions; but whenever a pattern tendency for lobing is put

into a cross with one of these species, the effect of it is clear. The result is seen in figure 7, where *Sanderæ alata* is crossed both with *paniculata* (12) and with *Langsdorffii* (9). One may note: first, the similar corolla lobing in the two hybrids; second, the size and shape of the tube, with individual influences for *paniculata* and for *Langsdorffii;* third, the difference in the

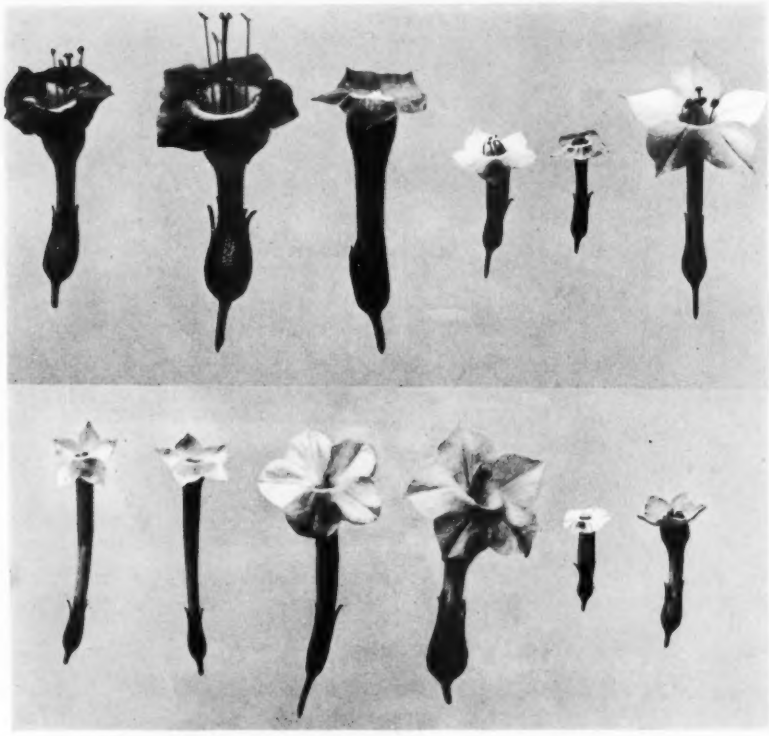

FIGURE 9.—Upper row: *N. Tabacum mac.×N. tomentosiformis; N. Tabacum mac.×N. tomentosa; N. Tabacum mac.×N. glauca; N. nudicaulis×N. tomentosa; N. nudicaulis×N. tomentosiformis; N. Bigelovii quad.×N. tomentosiformis.* Lower row: *N. glauca×N. longiflora plumbaginifolia; N. longiflora plumbaginifolia×N. glauca; N. suaveolens×N. glauca; N. glutinosa×N. sylvestris; N nudicaulis×N. trigonophylla (Palmeri); N. trigonophylla (Palermi)×N. tomentosiformis.* ×3/4.

amount of hairiness (the *paniculata* being practically without hairs); and fourth, the different effects produced by the decidedly different calyxes of *paniculata* and of *Langsdorffii.*

Figures 3 and 4 show the flowers of the hybrids and of the parents when *Bigelovii quad.* (24) is crossed with the three species *trigonophylla* (12), *suaveolens* (16), and *rustica* (24). The great limb spread in the hybrid

corollas, mentioned earlier, is the most obvious point to be observed. The size is even larger where *trigonophylla* (12) enters the hybrid than it is where *rustica* (24) enters. The individual influences of *trigonophylla*, *suaveolens*, and *rustica* are easy to detect, however, in the shape and form of the limb and in the shape and form of the calyx. It is less easy to see how *trigonophylla* influences the shape of the corolla lobes, but it is clear in the

FIGURE 10.—Upper row: *N. paniculata*; *N. paniculata*×*N. Tabacum mac.*; *N. Tabacum max.* ×*N. glauca*; *N. glauca*. Middle row: *N. rustica texana*; *N. rustica texana*×*N. Tabacum Cuba*; *N. Tabacum macrophylla*; *N. Tabacum mac.*×*N. tomentosiformis*; *N. tomentosiformis*. Lower row: *N. Tabacum Cuba*×*N. sylvestris*; *N. sylvestris*. ×3/4.

case of *suaveolens*. It is also clear in the slight lobing of the *rustica* hybrid, though the photograph does not show this characteristic very well.

Figure 5, the *longiflora* (10)×*Sanderæ* (9) hybrid, is a combination of members of two groups where the organ patterns are similar. The hybrid is consistently intermediate. In the photograph only the relative influences

of the two parents on size and shape of the tube are apparent; but in the living flowers, the influence of both parents on the texture is obvious.

One of the most interesting series (figures 5, 8, and 9) is the one illustrating hybrid unions made with *tomentosa* and *tomentosiformis*. In figure 9, upper row, flowers 1 and 2 are hybrids between *Tabacum macrophylla* (24) and *tomentosiformis* and *tomentosa;* while in the same row flowers 4 and 5

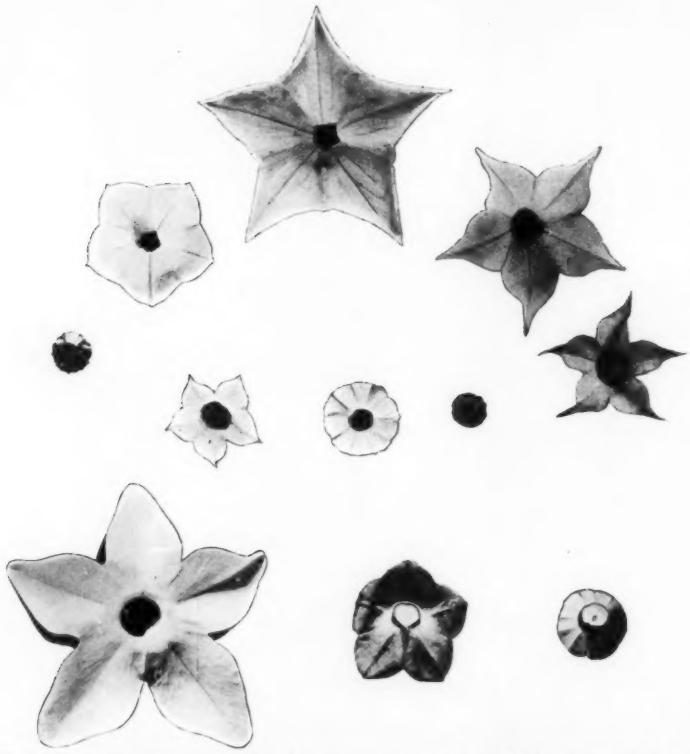

FIGURE 11.—Upper row: *N. paniculata; N. paniculata*×*N. Bigelovii; N. Bigelovii; N. Bigelovii*×*N. Tabacum lanc.; N. Tabacum lanc.* Center row: *N. Tabacum Cuba; N. Tabacum Cuba*× *N. paniculata; N. paniculata.* Lower row: *N. Sanderæ alata; N. Langsdorffii* ×*N. Sanderæ alata; N. Langsdorffii.* ×3/4. Except center row. ×9/16.

are hybrids between *nudicaulis* (24) and *tomentosa* and *tomentosiformis*. In both instances the hybrids are similar in character. The sole distinguishing feature is the greater size of the flower where *tomentosa* is used. This is the result one should expect from the two species, as may be seen by referring to the flowers of the two parental types in figure 8 (flowers 1

and 6, upper row). Such results, plus the fact that *tomentosa* and *tomentosiformis* give hybrids which are perfectly fertile, would appear to be conclusive evidence that the two are simply varieties of one species. One or the other of these varieties is also shown in combination with *trigonophylla* (*Palmeri*) (12), *glauca* (12), and *Bigelovii quad.* (24) in figure 8. The points to be noted are the specific influences of *trigonophylla* (*Palmeri*), *glauca*, *Bigelovii*, *nudicaulis*, and *Tabacum* on shape and constriction of calyx, length, and breadth of the tube, location and type of swelling of throat, type of limb, shape of petal lobes, and height of pistils and stamens.

From these same figures, one may also see the reactions of *glauca* with *tomentosa* (figure 8), *longiflora plumbaginifolia*, *suaveolens*, and *Tabacum*. Figures of the reciprocals between *glauca* and *longiflora plumbaginifolia* are given in order that it may be seen how nearly identical they are.

The last and largest series is the group of crosses with *Tabacum*, shown in figure 10. There are combinations with five other species. To these figures, three other crosses are to be added—those with *glutinosa* and *Bigelovii quad.* in figure 6, and that with *glauca* in figure 9. A flower of *Tabacum macrophylla* is figured in figure 10. But, unfortunately, though I have made the cross between *Tabacum macrophylla* and *sylvestris*, I have no photograph of it. The hybrid shown is with the *Tabacum* variety Cuba, where the flower is nearly like that of the Havana variety illustrated in figure 6, but with slightly deeper lobes. The lobes of the actual Cuba flowers are seen in figure 11.

It is regrettable that I obtained no photographs of *Tabacum* hybrids with *Sanderæ* and with *Langsdorffii*, the 9-chromosome group. The *Tabacum-Langsdorffii* hybrid had flowers approaching *Tabacum* in size and shape. They were approximately three-fourths size, with very little chlorophyll discernible. Of all our photographs, they were most similar to the *paniculata-Tabacum* hybrid, though smaller. The *Tabacum-Sanderæ* (*alata*) hybrid is figured in EAST and HAYES (1912). They were one-fourth longer than those of the *Tabacum* parent, with the limb reminiscent of *Sanderæ-alata*, but somewhat narrowed. The swelling at the throat showed *Tabacum* influence, as did the texture. The fresh flowers resembled *Tabacum* somewhat more than the illustration shows. One could imagine that if the hybrids had been made with a smaller-flowered variety of *Sanderæ*, they would have resembled *Tabacum* still more closely. And this was actually the case in a hybrid produced here by CHRISTOFF, though one could still call them intermediate.

In a casual examination, one would be likely to say that the hybrids with *Langsdorffii* (9), *paniculata* (12), and *glauca* (12), appear to stand very close to *Tabacum*, but this is only because the flower types of these three species have less to add in changing the form than have some of the

other species. The *Tabacum-Sanderæ* hybrid, where the pattern could be changed more abruptly, exhibited a marked influence by the 9-chromosome species. And one can trace the influence of the greenish-yellow flowered species in the calyxes and tube shapes of the hybrids. The influences of *glutinosa* and of *tomentosa* are observable in the zygomorphism of the flowers, in the length of the pistils and stamens, in the shape of the limb and the type of the corolla lobes, in the length of the tube, and particularly in the proportions of constricted and of expanded tube. In combinations with *sylvestris*, the other 12-chromosome species that is thought to be a component of the allotetraploid *Tabacum*, the influence of *sylvestris* is seen chiefly in the texture of the corolla, length of tube, the form of the swollen portion of the tube, and the constriction at the throat.

The very different influences exerted by *Bigelovii* (24) and *rustica* (24) in unions with *Tabacum* need no special emphasis. But it is very doubtful whether these species show more influence in the developed characters of the hybrids than do the species having smaller chromosome numbers, when the possibilities of changing the pattern are analyzed closely. *Glauca* and *paniculata* do not possess marked traits which can exert such influence. *Tomentosa*, *sylvestris*, *Bigelovii*, and *rustica* do possess characteristic patterns, and these patterns have their effect. To show what I mean by these statements, let us consider the two crosses with *paniculata* and with *rustica*. *Paniculata* and *rustica* have 12 and 24 chromosomes, respectively, and *paniculata* is a component of *rustica*. Moreover, they have somewhat similar flower patterns except for length and breadth of corolla. Does *rustica* appear to exert more influence on the form of the hybrid flower than *paniculata* in unions with *Tabacum?* After a casual examination, any observer would say that it does. But I believe that this is an illusion. The two hybrids both show the corolla limb of *Tabacum* with but slight modification. Where they differ is in the effect which *rustica* has in shortening and broadening the corolla tube. *Paniculata* has a slight swelling of the tube below the throat swelling. This is traceable in the hybrid. *Paniculata* has a more constricted throat than *rustica*. This pattern influence is also discernible in the hybrid.

Again, compare the combinations of *Tabacum macrophylla* with *glauca* (12) and with *tomentosiformis* (12). *Glauca* has a straight tube (the figure is slightly flattened), with a funneled, unlobed limb. The calyx tube is also straight. And both calyx tube and corolla tube are almost without hairs. The length of the flower is about the same as that of *paniculata*. One should expect that the hybrid, therefore, should resemble *paniculata-Tabacum*, as to size and shape, but with the calyx modified toward that of *glauca*. Hairiness should be restricted more than in *paniculata-Tabacum*, as *paniculata* has more hairs on the calyx, though the corolla is as smooth as *glauca*.

These modifications are precisely what are obtained. *Tomentosiformis*, on the other hand, has numerous identifying characters, which may be noted in calyx shape, sepal lobes, open throat with exceptionally marked swelling, peculiar pointed corolla lobes, and extruded stamens and pistils. There is an opportunity, therefore, for modification of the flower pattern in numerous ways. And all these modifications can be traced in the hybrids.

Or, one may compare the crosses of *Tabacum Havana* with *glutinosa* (12) and *Bigelovii quad.* (24), as shown in figure 6. Size relations are intermediate in both hybrids. But *Bigelovii* has a more hairy calyx than *glutinosa*. The calyxes of the two hybrids differ as expected in this respect. And *glutinosa* has a very hairy corolla, while *Bigelovii* is nearly smooth. The corollas of the hybrids exhibit this same reversal of conditions. The most conspicuous character of *Bigelovii*, however, is the funnel-shaped flower, while the most conspicuous character of *glutinosa* is the zygomorphic flower, with its peculiarly swollen throat. The hybrids stand intermediate with regard to these characteristics. *Bigelovii* has no more influence than *glutinosa*.

Thus one may analyze the entire series of hybrids; and, when this is done, an unexpected result is obtained. *All constituents of the pattern are active in modifying the pattern of the hybrids, and the grade of these modifications is little affected by differences in the amount of chromatin contributed.*

Figure 11 is given in order that the reader may trace modifications in the corolla limb. The cross between *Tabacum Cuba* and *paniculata* is figured because it illustrates one of the few cases where the use of two rather similar varieties of a given species gives different hybrid patterns. The corolla limb of the hybrid with *Tabacum Cuba* shows only a faint lobing, while that of the hybrid with *Tabacum macrophylla* (figure 10) shows considerable lobing.

Leaf reaction patterns

Leaf reaction patterns are traceable as the effects of the genom activity of the constituents of these hybrids just as are the flower reaction patterns, but several matters must be taken into consideration if one is to get a clear idea of the situation. The size of the leaves is influenced markedly by external conditions. Leaf-size is also influenced decidedly by internal conditions. If the hybrids are vigorous, the leaves may be large; if the hybrids are weak, the leaves may be small. It seems as if the machine does not run properly in certain combinations. It is also to be noted that the determination of sessile or of petioled leaves appears to be controlled by relatively few genes, as in varietal crosses between sessile and petioled *Tabacum* varieties. The reaction pattern that seems to be comparable with the flower patterns is that which controls the shape or proportions of the

leaves. Evidence that this is true appears in figures 12–16, which were kindly drawn from life, under my supervision, by my assistant, Miss ESTELLA HUMPHREY. Relative size is merely indicated.

Figure 12 illustrates two hybrids between *paniculata* (12), and *glutinosa* (12) and *rustica* (24). The *paniculata-glutinosa* hybrid shows the influence of both parents in the proportions above and below the point where the broadest part of the leaf comes, and also in the shape at the base. The

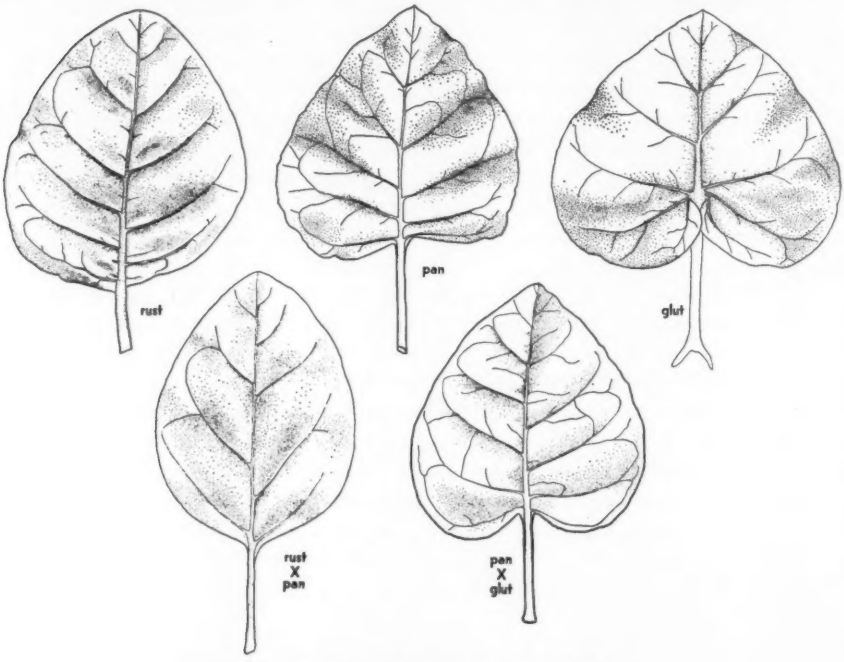

FIGURE 12.—Leaves of crosses with *N. paniculata*.

rustica-paniculata hybrid is somewhat more like *rustica* than it is like *paniculata*. This is particularly true at the base, where there is no tendency toward a cordate condition, and also in the texture, which is thick and smooth like the *rustica* parent. There is a tendency, however, for the broader part of the hybrid leaf to be shifted somewhat toward the petiole, as in *paniculata*. This tendency is not difficult to see when a goodly number of plants is examined, though it is not so obvious in the leaf from which the drawing is made. The latter is as close to the modal condition as I could find.

Figure 13 pictures the leaves of three hybrids made with *suaveolens* (16)

as one of the parents. The other parents are *longiflora* (10), *glutinosa* (12), and *Bigelovii* (24). The modification toward an obovate leaf, through the influence of *longiflora*, is easily seen. It is also obvious that the effect of *glutinosa* has been to shift the point of greatest breadth well toward the base of the leaf. The hybrid has a distinct petiole, though the proportion of blade to petiole has been increased by the influence of *suaveolens*. The base is presumably what one should expect in a combination of the tendencies of the two parents. The extraordinary thing in this figure is the

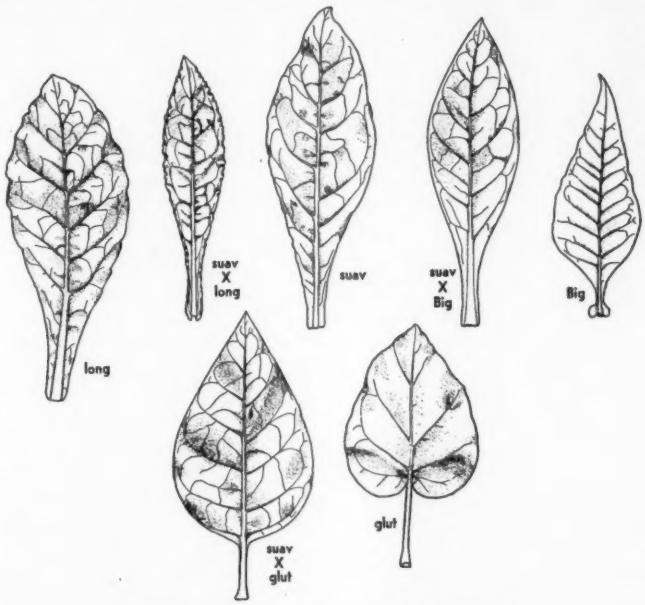

FIGURE 13.—Leaves of crosses with *N. suaveolens*.

apparent lack of influence of the 24-chromosomed *Bigelovii* in its combination with *suaveolens*. *Bigelovii* has a very striking type of leaf, ovate with a constricted base possessing auricles. The leaf of the hybrid is very nearly like that of *suaveolens*, though intermediate in texture and having the disagreeable odor of *Bigelovii*. I have no explanation for this exception to the general rule. It matches the *paniculata* situation in the other direction.

Figure 14 shows the leaves of four hybrids where *paniculata* (12) was combined with species having sessile leaves. The other parents are *Langsdorffii* and *Sanderæ alata*, with 9 pairs of chromosomes, and *Bigelovii* and *Tabacum*, with 24 pairs of chromosomes. Both *Langsdorffii* and *Sanderæ*

alata are markedly rugose, with great fullness of tissue between the veins. *Paniculata* is relatively smooth in this respect, although covered with downy hairs. The hybrids are intermediate in both of these conditions. *Langsdorffii* differs from *Sanderæ alata*, however, in having a blunter tip. Comparable leaves of these species also show that the latter commonly have a greater proportion of narrowed blade than the former. The hybrids show the specific influence of the 9-chromosome parents in both of these

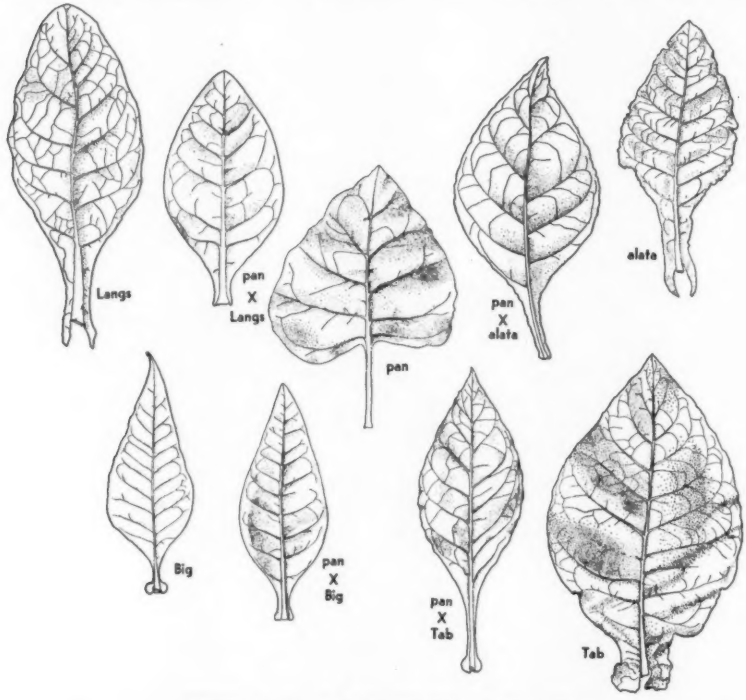

FIGURE 14.—Leaves of crosses with *N. paniculata*.

respects. The *Bigelovii* hybrid apparently resembles *Bigelovii* more than *paniculata* in shape and in basal constriction, though auricles are less in evidence. It should be noted, nevertheless, that though *Bigelovii* and *paniculata* are vastly different in relative length and breadth, they are rather similar in the proportionate amount of blade above and below the greatest breadth, if one imagines the proportions of *paniculata* drawn out into a sessile leaf. One may think of the sessile condition of the *Bigelovii* leaf as being partially dominant in this cross, while the petioled condition of *paniculata* is partially dominant in the *Tabacum* cross.

In figure 15, the leaves of parents and hybrids are reproduced for five crosses with the smooth, glaucous, petioled *glauca*. The other parents are *Langsdorffii* (9), *longiflora* (10), *glutinosa* (12), *tomentosiformis* (12), and *suaveolens* (16). None of the hybrids had bloom on the leaves, though where the texture of the leaf-tissue in the two parents differed, the hybrids were intermediate.

The leaves of *Langsdorffii* and of *longiflora* are superficially rather

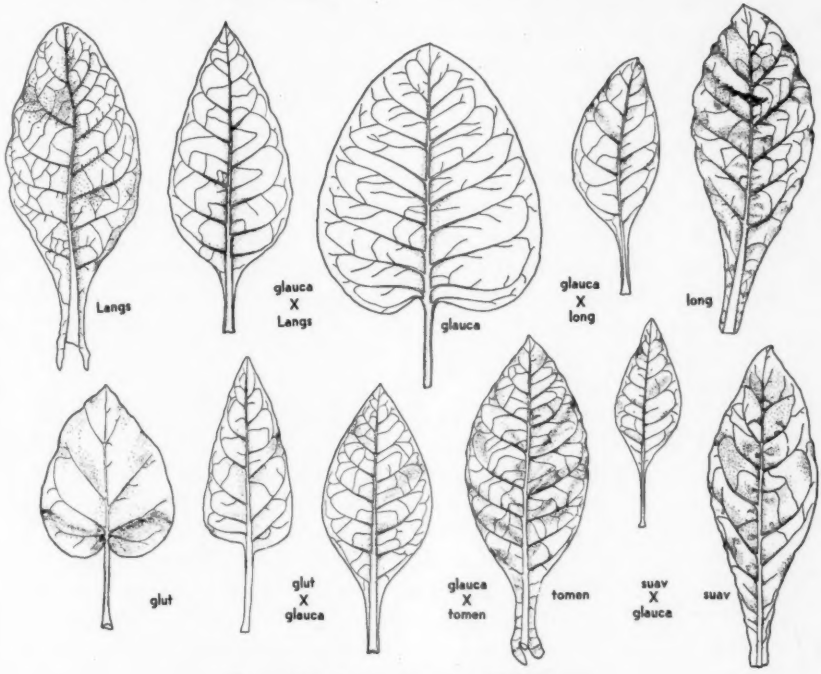

FIGURE 15.—Leaves of crosses with *N. glauca*.

similar. Both are rugose. They differ especially in three characteristics: *Langsdorffii* is soft, downy-haired, and has almost an ovate leaf, if one disregards the narrower part; *longiflora* is harsh, prickly-haired, and has the greatest breadth somewhat further toward the tip. *Glauca*×*Langsdorffii* leaves have soft hairs, particularly when the leaves are young. They are attenuated more nearly to a petiole than *Langsdorffii*, and have the relative proportion of blade from the tip to the greatest breadth intermediate between the characteristics of the parents. *Glauca*×*longiflora* leaves have a few prickly hairs. They have a goodly portion of the total leaf length attenuated toward a petiole. And the relative proportion of

the blade from the tip to the greatest breadth is less than in the other hybrid (influence of *longiflora*).

The *glutinosa-glauca* hybrid has something of a type of its own, as if it had been stretched out. The part having the greatest breadth comes at about the point one should expect, but the leaf then narrows unexpectedly. There is no trace of the cordate condition of the two parents. This condition is not inexplicable, however, if one considers the veination of the two parents. *Glauca* is pinnately veined; *glutinosa* has a tendency toward radiate veination at the base, which gathers the tissue of the base into an extremely cordate form. Now if the effect of *glauca* on the leaf pattern is to draw out the radiate veination at the base into more of a pinnate form, the result is that presented in the hybrid.

The influence of both parents is obvious in the leaves of *glauca*×*tomentosiformis*. It is also traceable in those of *suaveolens*×*glauca*. But there is less influence of *suaveolens* in determining the point of greatest breadth than one would ordinarily expect. The leaf-tissue, however, is narrowed to a fringed petiole throughout over one-third of the total length. This tendency exists in *suaveolens*, and of course there is a true petiole in *glauca*. Taking the total proportions into consideration, therefore, one can see that the point of greatest breadth is actually shifted toward the tip by the influence of *suaveolens*.

Figure 16 illustrates various crosses made with *Tabacum*. The variety of *Tabacum* illustrated is Cuban, as four of the seven crosses represented were made with that variety. In the other three crosses, with *glutinosa*, *paniculata*, and *sylvestris*, respectively, the *Tabacum* variety used was Havana. Any one interested can find a photograph of a Havana leaf (together with a Cuban leaf) in HAYES, EAST and BEINHART (1913) on Plate XII. The Havana leaf is longer than the Cuban, and the place of greatest breadth is a little nearer the tip.

Four of these crosses are with species having petioled leaves, where the influence of the members of the parental pairs is not easy to analyze. I may point out, however, that the reactions of these four petioled types, *paniculata*, *glauca*, *glutinosa*, and *rustica texana*, with the sessile *Tabacum*, are unlike. *Glauca*×*Tabacum* has a true petiole; *paniculata*×*Tabacum* has almost a petiole, but the pseudo-petiole is fringed to the base and there expands into noticeable auricles; *Tabacum*×*glutinosa* is sessile, though narrowed at the base like several of the other species; and *Tabacum*×*rustica texana* (24 chromosomes), though influenced by the *rustica* shape, is narrowed to a short fringed petiole, followed at the base by fair-sized auricles.

The influences of *Sanderæ alata*, *longiflora*, and *sylvestris* are traceable in the form of the patterns, if one considers particularly the shape of the

base and the relative length of the blade to the point where the greatest breadth is seen. Individual influence is still more obvious in matters of

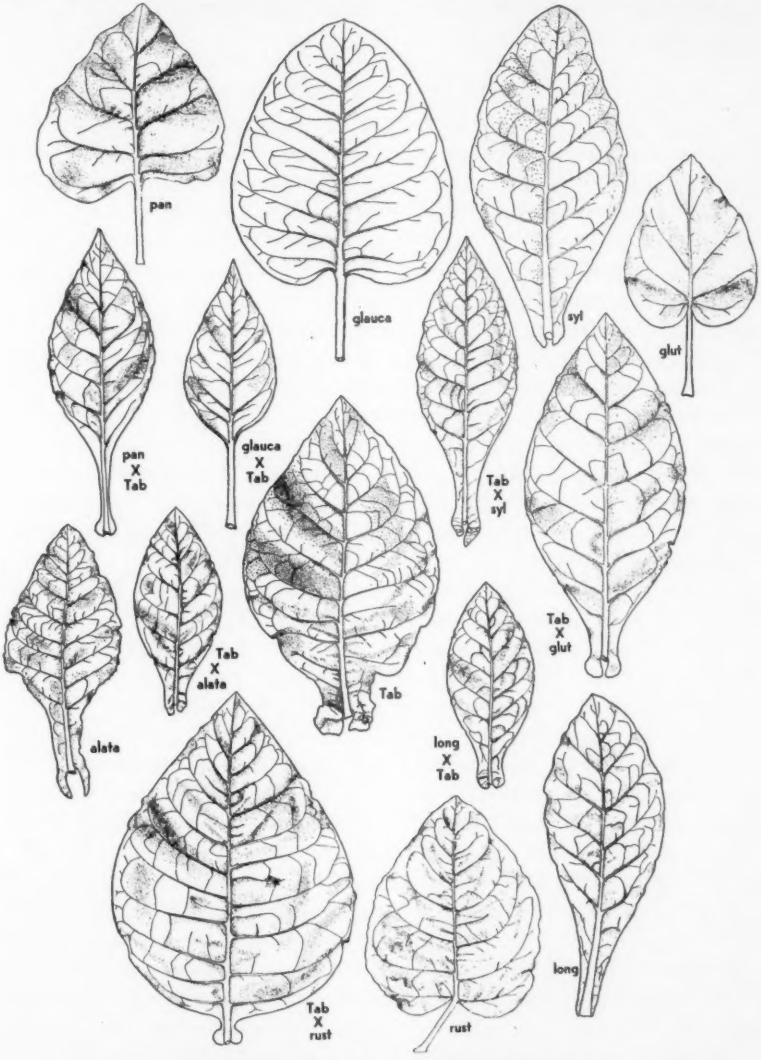

FIGURE 16.—Leaves of crosses with *N. Tabacum*.

texture, veination, color, rugosity, and hairiness, which are points that do not show in the illustrations. There appears to be no material quantita-

tive difference in the effects produced by the 9-chromosome species (*Sanderæ alata*), the 10-chromosome species (*longiflora*), the four 12-chromosome species (*glauca*, *glutinosa*, *paniculata*, and *sylvestris*), and the 24-chromosome species (*rustica*).

DISCUSSION AND SUMMARY

In this paper, data are submitted on about sixty hybrids between Nicotiana species. No attempt was made to describe each of these hybrids in minute detail, as might have been done had the objective been taxonomic. Such description as seemed essential has been given, plus photographs and measurements from which the reader may draw his own inferences, with the definite idea of trying to see whether useful conclusions could be drawn regarding the problem of phenotypic behavior in hybrid combination. The objective, in other words, is genetic. The treatment is the analysis of what occurs when a given species is crossed with several other species having the same or different chromosome numbers.

Four conclusions emerge. The first is reasonably conclusive; the other three, though somewhat heterodox, are strongly indicated.

1. On the basis of data from 15 pairs of genetically comparable reciprocals—that is, reciprocals made with identical pure parents or with sister plants belonging to near-homozygous lines—it may be stated that reciprocal hybrids in Nicotiana are as nearly identical as are plants in a pure line of a given Nicotiana species. This is true whether the parental forms have the same or different chromosome numbers.

In spite of the facts, therefore, that the genus has become highly differentiated and has developed forms having several different chromosome numbers, there is no evidence that the species have distinctive plasmons in the sense of VON WETTSTEIN.

2. The gene changes producing the qualitative effects, which differentiate minor varieties and are so largely used in genetic research, appear to be different, as a general group, from the gene changes which accumulate into specific differences. The latter are usually quantitative and are difficult of demonstration for this reason. The qualitative variations are usually easy to detect because they so often cause distortions, restrictions, or stoppage of what have come to be normal processes through natural selection. In their inheritance one finds these strange peculiarities: dominance, where A is sufficient to make the process go on almost as well as AA, but a restricts it; inhibitors; several genes necessary for the production of a detectable difference; and so on. Such genes, I am inclined to believe, are, by their nature, usually incapable of playing a part in natural evolution, though they may be very advantageous in building up knowledge of the heredity mechanism.

When such genic differences do not tend to depart too abnormally in their control of the life processes, they appear to be transmitted in species crosses as in variety crosses (color reactions). But this may not be the case if processes concerned happen to be of the more essential type that can be influenced materially by a large number of genes (sessile and petioled leaves). Here one finds phenomena such as FISHER imagined in his writings on the evolution of dominance. But the important point is that the effect of different residual germ plasm is detectable in various manners. There appears to be no effect of the type required to pass in one direction and evolve dominance, but rather an effect which produces a "pattern" that is different.

3. When one studies this series of hybrids by taking each species in turn and asking what is the effect on form when various other species are combined with it, one is considering combinations of gene processes that have survived natural selection. Each genom has possibilities of normal orderly processes rather than restricted, inhibited, inefficient processes. And when one does this, he is forced to the conclusion that the various genes of each genom are active in producing slight changes in developmental patterns, whether these patterns be in flowers, leaves, or habit of growth. There is, therefore, hardly any evidence of the phenomenon of dominance which is more or less characteristic of the other group of gene changes.

4. A detailed analysis of pattern effects gives *no* support to the idea that the influence of a genom is in some way proportional to its contribution of chromatin. Reactions are varied, it is true, but in a 9–24-chromosome combination, special effects are as likely to be exerted by the 9-chromosome parent as by the 24-chromosome parent. In the idea, as generally held, that chromatin has somewhat of a mass action, it would seem that there is a postulate of numbers of actively different genes in proportion to amount. It is obvious that this view is unsound if one stops to think a moment. A 9-chromosome *alata* plant has the ability to produce a complete Nicotiana with the special features of *N. alata*, just as the 24-chromosome *rustica* plant has the ability to produce a complete Nicotiana with the special features of *N. rustica*. The reason we get pattern effects here and dominant (often) effects in certain varietal crosses is, as I believe, inherent in the type of reaction.

The current view would probably not have been advanced if a reasonably large series of species hybrids had been analyzed in the manner suggested here.

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GENETIC REACTIONS IN NICOTIANA. III. DOMINANCE

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The title of this paper is a slight misnomer. The views to be presented on the relation of dominance to gene activity are thought to have general validity. Naturally, therefore, they have not been formulated without due consideration of published genetic records. My own personal genetic experience necessarily has been influential in their development, however, and much of this experience has been gained by the use of material from the genus Nicotiana.

The accepted facts regarding the phenomenon of dominance may be outlined as follows, the statements departing only slightly from those set forth by WRIGHT (1934). 1. Dominance and recessiveness are one-word descriptions of the appearance of end-products in the physiology of development, and have nothing to do with the distribution of genes to the filial generation. 2. The contrast that these words describe is one in which the recessive is usually a restriction or a failure of a positive property found in the dominant. 3. An approach to dominance of a given allele *A* over allele *a* is the rule with the pairs of genes ordinarily used in genetic work. 4. Mutant genes that have been detected and their resultant activities described under controlled conditions are overwhelmingly recessive to the genes from which they have arisen when crosses are made with the stocks from which they have originated. 5. Recessive genes, when homozygous, are usually less advantageous to the possessor than their dominant alleles. It follows that recessive genes are ordinarily found less frequently than their dominant alleles in natural populations, since, as WRIGHT states, deleterious dominant genes tend to be eliminated rapidly (frequency in population $q = \frac{u}{hs}$, where u is mutation frequency and hs is disadvantage of heterozygote), and advantageous ones preserved. Disadvantageous recessive genes, however, are kept at relatively higher frequencies in a natural population than are disadvantageous dominant ones ($q = \sqrt{u/s}$, where s is disadvantage of the homozygote). WRIGHT calculated that when the selective disadvantage of a homozygous mutant is .01 and the mutation frequency is 1 per million per generation, a deleterious recessive gene will be kept at 100 times the frequency of an equally deleterious dominant.

The hypotheses in which endeavors have been made to account for dominance and recessiveness have not been very satisfactory.

The first to gain recognition was the "presence and absence" theory of

BATESON and PUNNETT. Here it was assumed that the commonest type of mutation was a loss of some kind. If this were the case, it was thought reasonable that the loss should entail recessiveness on the ground that one dose of a gene having constructive activity should produce an end product more like that of two doses than like none at all. The more ferocious critics of this conception have imputed to BATESON and PUNNETT the crude idea of an absolute physical loss, but I have always felt that this did not do the authors justice. What they really had in mind, I believe, was that the dominant gene can do something that the recessive gene can not do, and in this sense they approached the truth. Nevertheless, the presence and absence theory has been discarded by geneticists for various reasons. It was difficult to visualize evolution by losses, to account for reverse mutations, to explain certain multiple allelic series, or to see how certain apparent losses could result in dominant effects.

FISHER (1928 and later) has built up a theory of dominance which has only one physiological implication, namely, that the heterozygote is inherently more modifiable than either homozygote. FISHER simply accepts without question the postulate that nearly all mutations are recessive to wild-type. If one is to accept evolution, therefore, dominance must be evolved from recessiveness. This is done by assuming a continued selection of heterozygotes toward resemblance to wild-type by the collection of modifying genes.

It may be noted here that during the mathematical studies on evolution that FISHER has carried out, he found that the known mutation frequencies in various organisms are wholly insufficient to account for known results of the process. I believe that this observation is more important than the dominance theory, although the latter has received wide acclaim while the former has remained unnoticed.

WRIGHT has criticized FISHER'S theory, on the ground that any conceivably sound selection pressure would be too small to do the work assigned to it. HALDANE (1930) has made a similar criticism. The essential point in these criticisms is that the modifiers may have selection pressures acting on them individually which will necessarily take precedence over effects on the so-called primary genes present in the rarer heterozygote. WRIGHT has "emphasized more the point that even in those cases in which the homozygote is not capable of being modified in the same respect as the heterozygote, the modifier is likely to have other effects subject to direct selection." HALDANE has stressed the point that the same reasoning which leads one to believe that a given gene will modify the heterozygote, also leads one to believe that it will modify the wild-type.

PLUNKETT (1932) has drawn attention to the fact that if there is direct

selection of a type, this would tend to build up a factor of safety insuring an organism against variation in general.

GOLDSCHMIDT (numerous papers) and WRIGHT (1934) have endeavored to build up an idea of dominance based largely on what they conceive to be the physiology of developmental processes. GOLDSCHMIDT stresses quantitative action of catalyzers entering into action at specific times as development proceeds. WRIGHT also uses the catalysis idea, but brings it to bear specifically on the dominance question rather than on general development. He postulates that the most active phase of a gene is dominant over less active phases. Partial dominance, he feels, implies that "variations of the gene and its successive products leading to the observed character *all* affect the limiting components in the reactions into which they enter." "If there is still incomplete dominance at a stage in the reaction chain at which an all-or-none reaction intervenes, dominance of a gene comes to depend on the effects of independent modifiers and in this case is without relation to its primary activity."

I feel that each of these writers is reaching toward the truth. There is something worth-while in the presence and absence theory when stated in a manner in keeping with modern genetic knowledge. Modification of a given effect A can be changed by the action of accessory genes (see "Genetic reactions in Nicotiana. II," for reactions on petioled and sessile leaves), though I do not feel that this process is important in evolution. Chain reactions are the rule in development; and naturally, mass action, all-or-none reaction, and reactions of numerous other types must enter. One can get a very fair theoretical idea of the situation, provided one does not try to explain everything on a single mechanism or process.

Several years ago, I became convinced that certain modifications of our ideas on the nature and frequency of mutations are necessary before we can obtain a clearer view of these matters than now prevails. I believe that sufficient evidence exists to support these changes of view. If this is granted, a number of puzzling problems no longer present difficulties. These theses will follow. They are stated categorically, though exceptions must be recognized.

1. *The great bulk of the mutations detected in the genetic laboratory and used in genetic research are recessive to the normal, the wild-type, or the survival type, as it may well be called, because they cause restrictions in the physiological processes in which they are involved.*

It may be assumed that a high degree of efficiency in carrying out essential life-processes is called for in types of organisms that have survived the sieve of natural selection. The basis for these developmental processes lies in the bundle of genes that the individual inherits from its

parents. Each gene has an indispensable function to perform, if one may judge from the fact that deficiencies for single genes always appear to be lethal when in the homozygous condition; but it is not necessary to assume that the efficiency rating of each and every process must be similar. Now, if we look upon the gene as a chemical entity, or a chemical factory—if need be—it is clear that on chemical analogy a limited number of changes is to be expected. It is convenient to denote these changes, or mutations, by the term "genic isomers," without wishing to stretch the chemical simile to the breaking point. These changes we may expect to be quite limited on any imaginable biochemical view and because of the facts as we have them in the genetically well-known organisms such as *Drosophila melanogaster* and *Zea Mays*. It must be remembered, however, that the facts, as they are usually listed, may give a distorted view of the truth. When the wild-type eye of Drosophila mutates to eosin in various stocks, they are recorded together to make up an eosin mutation frequency; but there is no way by which to be certain that two or more eosins (that is, different genic isomers) giving similar results are not included. TIMOFÉEFF-RESSOVSKY (1932) has recognized this problem and has tried to throw some light on it through studying mutations and inversion frequencies in material from various sources.

It is probably to be expected that the great bulk of the possible genic isomers result in physiological restrictions of the normal (selected) processes, though this is not necessarily so. At all events, the mutations producing physiological restrictions are the ones easily detected, just as the faults of a car which cause missing cylinders, et cetera, are easier to detect than the vague matters which make it run a little better than it did yesterday. If the process needing the gene's activity comes to a complete stop, the condition is lethal. If, on the other hand, it limps along inefficiently, the organism will live, though the principal end product by which genes are named will not be the same as in the normal.

It is easy to see that, on the view that numerous mutations are in the nature of physiological defectives, several conclusions follow that are borne out by experience. Physiological defective a, unless disturbing to the activity of the normal A, will be recessive to a degree proportional to the defectiveness; for, on any reasonable biochemical view of the gene as a working laboratory, the activity of one normal gene should give an approach to a normal product. This type of mutation should often be lethal or deleterious in the homozygous condition; but if the main activity of the gene is the production of non-essential products (as flower colors), it need not be so. Such genes should be readily detectable because commonly the activity of defective genes should result in end-products that are qualitatively different from those produced by the normal.

2. *"Defective" genic isomers are not the mutations that serve as material in evolution.*

FISHER found it necessary to formulate a theory of the evolution of dominance because he assumed that the mutations described for Drosophila gave a true picture of the mutations available for the differentiation of forms. If the assumption is false, the theory is unnecessary. I feel convinced that it is false.

It is unnecessary to labor the defense of this point. The place to seek the kind of material useful in the origin of species is in Nature rather than in the genetic laboratory. Let the taxonomist be the judge. And taxonomists, whether they deal with animals or plants, whether they consider the so-called lower or the higher forms, are unanimous in maintaining that the type of alternative characteristic ordinarily utilized in the genetic laboratory for the study of heredity is useless in discriminating between species. Species commonly differ in a lot of quantitative relations, in patterns. The beginning of species differentiation may be visualized as change in a few of these quantitative relations.

These quantitative changes (there may also be occasional qualitative changes) in the end results that we know as characters, require the accumulation of numerous "gene change" effects in order to be recognizable. The mutation in a single gene, therefore, is not readily detectable. Even many such changes resulting in describable end results, are difficult to deal with genetically. This is the reason why investigators familiar with wild forms that will cross together and produce fertile hybrids have found difficulty in interpreting their results. As competent an investigator as SUMNER, for example, worked for years with Peromyscus before he became convinced that inheritance in this genus is Mendelian. The reason for his hesitation is understandable. He was dealing with the kind of situation that we are discussing.

These mutations, as I have said, commonly produce results that may be said to be quantitative. Such a description is not wholly satisfactory, however, for there may be many exceptions. What I feel to be a truer conception, though one that is difficult to prove conclusively, is that the gene changes which give these results are physiologically non-defective. Perhaps an example will serve to show the distinction. The ligule is characteristic of the entire group known as the Gramineae. Liguleless stocks are known in maize, rye, and oats. And the two conditions behave as if controlled by a single pair of genes. Now, although a liguled type may give rise to a liguleless type by one mutation, it is not reasonable to assume that liguled forms arose from liguleless stocks in a similar way. It is more in keeping with our general knowledge to assume that the ligule is an organ having a long evolutionary history, for it exists in numerous forms. It pre-

sumably is the result of a very large number of non-defective mutations in various genes, and, physiologically speaking, is the end product of a long chain of reactions. But a single defective change breaks this chain and gives a liguleless plant. The building up of the normal mind, contrasted with the single defective mutation that entails feeble-mindedness, is an even better example.

3. *Non-defective gene mutations are frequent in Nature, but are difficult to detect.*

I believe that every experienced plant breeder will agree with this statement. It will accord with his experience. I will relate some of the experiences that have led me to the conclusion.

During the early years of my Nicotiana investigations, over 100 varieties of *Tabacum* and *rustica* were studied. Some were discarded after one population had been grown; others were observed in selfed lines over long periods (maximum 28 years). Numerous crosses were made between commercial types of *Tabacum* and, to a less extent, between commercial types of *rustica*. Several studies, involving hereditary differences in height, number of leaves, and size, shape, and texture of leaves, were published by HAYES, JONES and EAST. It is not necessary to discuss these reports beyond saying that the selection experiments on these quantitative characteristics, such as those published by EAST and HAYES (1914), were carried on to the F_{13} generation and were evaluated statistically, though not published. They are mentioned for one purpose only. This very considerable experience with continuously self-pollinated lines of supposedly pure types and with successively selected self-fertilized lines from varietal crosses indicated very strongly that though there is a rapid approach to homozygosis of gross characters, there still remains a large amount of apparently irreducible variability in all parts of the plant, some of which can be proved, by appropriate tests, to be heritable. For example, the tobacco leaf varies in characteristic items of shape from the tip to the base, in color, in size, in veination, in fullness between the veins, in thickness, and in various other factors that go to make up commercial quality after what is ordinarily thought to be a pure type is obtained. In selected descending self-fertilized lines, one may obtain a still more uniform progeny temporarily, as regards almost any peculiarity, thus showing a residuum of heterozygous genes affecting these characteristics. But there must be a high mutation frequency since, after 10 or 12 generations of closest inbreeding, one can not reduce, in an appreciable degree, the variability of any indices for the leaf as a whole.

In spite of the effect of environmental differences on the plants of such populations, and in spite of the limited validity of such observations due to errors of sampling, the uniform nature of such experiences leads me to

believe that in organisms currently undergoing a fairly rapid evolution (and most Angiosperms probably belong in this group), gene mutations are constantly occurring with many times the frequency that is generally assumed. The reason for this discrepancy lies in the ease with which one can detect the physiologically defective mutations, the changes which throw sand in the life machinery—as one may designate 99 percent of the Drosophila mutations—and the difficulty of detecting slight genic isomers where the results must usually be expressed by ideas of plus and minus.

This conclusion was strengthened some years later by another series of experiments, though I did not appreciate their significance at the time, and therefore can not support them quantitatively. While I was endeavoring to produce certain specific hybrids, numerous combinations were observed which yielded only maternals. Nearly all the plants were ordinary fertile diploids, though occasionally (perhaps 1 per 1000) smaller sterile plants occurred that must have been haploids. Afterwards it was proved, first on Fragaria (EAST, 1930) and later on Nicotiana, that these maternals came from mature gametes in which parthenogenetic development had been induced. There were several reasons for supposing that diploidy came about at a very early embryonic division, and therefore that each plant was a complete homozygote. Since the immediate populations in such cases are simply random samples of the gametes of a given mother plant made diploid by mitosis (the capsules often being full), there was nothing noticeably peculiar about them. But several species were continued from such plants self-fertilized, *N. rustica* furnishing the most extensive series of observations. Over a dozen different progeny rows of *rustica*, for example, gave a somewhat astonishing result. The plants of each progeny test were as much alike as if they had been cut with a die. The condition was so noteworthy that I tried, without very great success, to make photographs showing the uniformity. A few measurements of various characteristics were taken, but they gave too much of a partial picture of the situation to be of value. One may rest assured, however, that the individuals in each of these populations were more strikingly similar than those of *any* ordinary inbred populations that I had ever examined. And now comes the feature to which I wish to draw particular attention. Several of these lines were continued from self-fertilized seed, and within 3 or 4 years they were showing approximately the same amount of variability as ordinary selfed populations, a result which could only have come about from frequent small mutations.

Attention should also be called to the minuteness of the parental influence on the hybrid patterns of any plant organ, as shown in the analyses made in "Genetic Reactions in Nicotiana. II." It is difficult to explain these influences unless one assumes that during the differentiation of the

Nicotiana species a large number of non-defective mutations have occurred at various loci. It is clear, however, that individually they would be difficult to detect.

If the truth of theorem No. 3 is granted, FISHER'S requirement of a higher degree of mutation frequency than is shown by calculations made from the defective gene mutations of Drosophila is satisfied.

4. *Non-defective gene mutations, though distributed in the ordinary manner, operate in such a way that the result of their activities is not ordinarily recessive to wild-type.*

There is no reason to believe that non-defective gene mutations may not sometimes show an approach to dominance and sometimes an approach to recessiveness to wild-type, but the characteristic of a non-defective gene is its physiological constructiveness. The characteristic of the genes with recessive tendencies is physiological destructiveness. The allelic red eye-white eye series in Drosophila, for example, I should regard as a physiologically destructive series in which recessiveness is directly proportional to amount of defectiveness. The agouti series in rabbits, on the other hand, may possibly be a non-defective series, though I do not so regard it. It is mentioned only because similar series appear to be found in the wild and therefore could be considered as a hypothetical example of a non-defective series where there is partial dominance of higher degrees of pigmentation over lower degrees. I also wish to point out that the recognized agouti series is such only because of detectable differences between the members. There may be numerous other grades that are not detectable. Mammalian geneticists have suspected that they exist.

The experiences from my own work leading to the formulation of theorem No. 4, however, come largely from a consideration of the species hybrids in Nicotiana. Naturally one must admit that here the situation is complicated because of the existence of numerous genic differences. Nevertheless, when one considers the minuteness of detail in which one can trace the influence of both parents in forming an intermediate pattern in habit of growth, type and form of leaf, hairiness, chlorophyll production, and size and shape of all flower parts, it would seem that the theorem is valid.

5. *The deviations forming the fundamental material of evolution are the small variations of* DARWIN.

This conclusion follows as a matter of course. We return to the Darwinian idea, modified by the demonstration of alternative inheritance.

SUMMARY

In this paper there has been an attempt to show: (a) that most recessive genes are genic isomers that are defective physiologically and are easy

to detect on this account; (b) that such mutations are not the material that is useful in evolutionary processes; and (c) that non-destructive mutations occur with a high degree of frequency, that are not ordinarily recessive or dominant to wild-type but are active pattern formers just as are wild-type genes, and are difficult to detect on this account.

It is thought that the recognition of this class of mutations gives geneticists a new orientation to the problem of evolution. If such mutations occur with the frequency which certain evidence obtained from species hybridization experiments and from selections experiments suggests, the mathematical requirements for evolution under alternative inheritance may be satisfied, and theories as to the evolution of dominance made unnecessary.

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THE VARIATION OF TWO SPECIES OF LYMNAEA

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The problem of the effects of isolation in relation to the establishment of subspecies and local races has been studied by many workers since the time of DARWIN and WALLACE, but in most instances it has been attacked only from the standpoint of geographical speciation. Little attention has been paid to the relationship existing between the type of variation in any one circumscribed locality, and that occurring over extensive geographical areas. The naturalists of sixty years ago were interested in the gross effects of isolation, as exemplified, for instance, in the fauna of oceanic islands, but paid no great attention to the possibility of studying this phenomenon over broad and continuous areas. The work presented in this paper covers an extensive territory in the Sub-Arctic region, and in certain respects runs parallel to the extensive researches of CRAMPTON (1916) upon the members of the genus Partula in certain Pacific islands, but with the important difference that CRAMPTON'S work covers a very small subtropical area. It appears that the differentiation of new species and races takes place in the first-mentioned region at a much slower rate than in that with the warmer climate, and this renders it possible, in certain respects, to follow the process with more precision. The analysis of this phenomenon in the northern part of North America may therefore be of interest.

The ideal animal with which to commence a study of this kind is one which has a very wide geographical range, a considerable tolerance of diverse environmental conditions, and a marked tendency toward variation. For comparison with this it is desirable to use a species which, while equally variable in form, is more specific in its habitat requirements. These conditions are admirably fulfilled by two species of fresh water mollusca, namely, *Lymnaea palustris* (MULLER), and *L. emarginata* Say. *L. palustris* is a hardy and widely distributed snail which is found in North America, Europe, and northern Asia; while *L. emarginata* is much more restricted in its range, being found only in the northern part of North America east of the Rocky Mountains. A comparison of the local and geographical variation of these two species shows that while in *L. palustris* the range of variation in one locality approximates that which occurs over almost the whole range of the species in North America, in *L. emarginata* there is a well marked tendency for the range of variation in any one locality to constitute only a small part of the range which is to be found over the whole

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geographical area inhabited by this species. That is to say, in the one instance local variation approximates geographical variation in range, while in the other it covers only a small part of it. In other words *L. emarginata* tends to split up into local races, whereas *L. palustris* does not.

THE SYSTEMATIC POSITION OF THE SPECIES STUDIED

The species dealt with in this account belong to the family Lymnaeidae, which in its old usage was believed to include not only the genus Lymnaea, but also Planorbis, Planorbula, and similar forms which are now placed in separate families. When the group came to be studied intensively in modern times, many new species were described, former varieties and subspecies were looked upon as species, and larger groups which had previously been regarded as sub-genera were raised to the rank of genera. As a result of this the name Lymnaea has tended to disappear from conchological literature in recent times, being supplanted by such names as Stagnicola and Fossaria, which while they may be more acceptable to the specialist, have the disadvantage of being less familiar to biologists in general. However, throughout these changes in nomenclature the relative status of the two groups designated here as *Lymnaea palustris* and *L. emarginata* has remained fairly constant, and there can be little doubt that they represent fundamental natural groups of the North American mollusc fauna. [BAKER (1934) considers that certain of the specimens here included as extreme variants of *L. palustris* are a new species.] Whether they are termed species or genera is of no great practical importance in the present connection. The animal referred to as *L. palustris* (MULLER) has been called *L. vahlii* ("BECK" MÖLLER) in a number of publications (MOZLEY 1934), but it now appears to be desirable to revert to the more general name, *palustris*, of MULLER. The diverse forms of the species here called *L. emarginata* have been referred to by various names, but with the possible exception of certain races which approach *L. catascopium* their close mutual relationship is generally admitted by conchologists. The significant fact in this connection is that *L. palustris* and *L. emarginata* are well marked species, absolutely distinct from one another, and are not readily mistaken for any other members of the North American fauna.

MATERIAL AND METHODS

The material upon which this study is based is for the most part from my own collection, which is now in the UNITED STATES NATIONAL MUSEUM. The shells of many of the series have been individually numbered, so that it will be a relatively simple matter for later workers to refer to them in the museum collection for purposes of confirmation or making additional observations. In accordance with the scheme for the preserva-

tion of biological statistics too voluminous to permit of publication, a copy of the measurements which form the basis of this work will be deposited with the Secretary of the ROYAL SOCIETY OF EDINBURGH.

LIST OF THE SERIES OF SHELLS MEASURED

Lymnaea palustris

NO. OF SERIES	LOCALITY	NUMBER OF SPECIMENS MEASURED	MEAN LENGTH OF SHELL
1.	Greenland	2	11.4 mm.
2.	Bernard Harbour	4	11.7
3.	Dall River, Alaska	2	12.0
4.	Fort Resolution	14	12.4
5.	St. Michaels, Alaska	24	12.4
6.	Yukon River	1	13.0
7.	St. Michaels, Alaska	2	13.2
8.	near Clair, Saskatchewan	4	13.6
9.	Little Quill Lake, Saskatchewan	50	14.0
10.	5 miles west of Fort Yukon	1	14.0
11.	Moose River, Ontario	2	14.1
12.	Paskwegin Brook, Saskatchewan	27	15.7
13.	Ironspring Creek, Saskatchewan	8	15.8
14.	Lake Mildred, Alberta	67	16.1
15.	Van Patten Creek, Saskatchewan	17	16.1
16.	Fishing Lake, Saskatchewan	20	16.5
17.	Atlin, British Columbia	7	16.5
18.	Milligans Creek, Saskatchewan	9	17.4
19.	Bernard Harbour	5	17.6
20.	Southern Twin Lake, Alberta	209	17.7
21.	Lake Mildred, Alberta	7	17.9
22.	Clair Brook, Saskatchewan	50	17.9
23.	The Grande Marais, Manitoba	29	17.9
24.	Atlin, British Columbia	2	19.6
25.	Shonts, Alberta	271	20.7
26.	Molson, Manitoba	6	21.0
27.	Duck Mountain, Saskatchewan	100	22.0
28.	Birtle, Manitoba	47	22.3
29.	Kelliher, Saskatchewan	25	24.3
	Total number of shells	1012	

The shells of these gastropod molluscs are convenient objects for measurement, and the dimensions taken as standard for this work are those which are almost universally adopted by conchologists, and may be briefly defined as follows:

Length of shell—the distance from the apex to the lower edge of the lip.

Greater diameter—the greatest width of the shell as a whole, measured along a line at right angles to the axis of the shell, and in the same plane as the aperture.

Lesser diameter—the width of the shell on a plane at right angles to that of both the axis and aperture.

Aperture length—the length of the orifice along a line parallel to the axis.
Aperture width—the width of the orifice along a line at right angles to the axis.

Measurements of these five dimensions were made upon 1012 shells of *Lymnaea palustris* from the twenty-nine localities listed on page 454. Twenty-six series of *L. emarginata* were measured, the number of specimens being 452. The number of specimens in a series from a single locality varied from one to two hundred and seventy one.

Lymnaea emarginata

NO. OF SERIES	LOCALITY	NUMBER OF SPECIMENS MEASURED	MEAN LENGTH OF SHELL
1.	Grand Rapids of the Athabaska, Alberta	16	12.8 mm
2.	Aroostook River, Maine	8	13.8
3.	Lake Winnipegosis, Manitoba	31	14.3
4.	Eagle Lake, Minnesota	6	14.5
5.	Cross Lake, New York	12	14.9
6.	Rainey Lake, Ontario	40	15.4
7.	Madison, Wisconsin	6	16.5
8.	Douglas Lake, Michigan	12	16.7
9.	Sault Sainte Marie, Michigan	2	16.7
10.	Madison County, Montana	7	17.0
11.	Hell Gate, Montana	6	17.3
12.	Milwaukee, Wisconsin	3	18.6
13.	Victoria Beach, Lake Winnipeg, Manitoba	35	19.3
14.	Fish River, Maine	5	19.6
15.	Crystal Lake, Minnesota	20	20.2
16.	Lake Constance, Ontario	3	21.2
17.	Atikameg Lake, Hudson Bay Railway, Manitoba	100	21.9
18.	Douglas Lake, Michigan	5	22.1
19.	Crooked Lake, Michigan	5	22.4
20.	Bear Lake, Michigan	10	22.7
21.	Clear Lake, Riding Mountain, Manitoba	90	24.0
22.	Lake Michigan	6	24.4
23.	Brome Lake, Quebec	4	24.7
24.	Lake Higgins, Michigan	13	25.9
25.	Square Lake, Michigan	3	27.2
26.	Lake Houghton, Michigan	4	28.7
	Total number of shells	452	

A convenient means of gauging the extent of variation in a series of shells lies in a comparison of the ratios of certain dimensions. The particular ratios here adopted for this purpose are the following:

$$\frac{\text{Length}}{\text{Greater Diameter}}, \frac{\text{Length}}{\text{Aperture Length}}, \frac{\text{Aperture Length}}{\text{Aperture Width}}, \frac{\text{Greater Diameter}}{\text{Aperture Width}}$$

It has been found that there is little change in these ratios during the latter part of the life of the animal.

TABLES OF FREQUENCY DISTRIBUTION

The accompanying tables, which show the frequency distribution of the dimensional ratios mentioned above, are a convenient means of gauging the range of variation in the series of shells from different localities, and when all four ratios are considered together, they may perhaps be taken as an expression of the variability in growth of the organism as a whole.

In preparing the material for publication it has been considered desirable to present the data in as simple a form as possible. In the tables of frequency distribution each unit represents an individual animal, in which the proportion of the shell dimensions is of the magnitude indicated. The

LYMNAEA PALUSTRIS

LENGTH / GREATER DIAMETER

SERIES NO.	1.60 TO 1.64	1.65 TO 1.69	1.70 TO 1.74	1.75 TO 1.79	1.80 TO 1.84	1.85 TO 1.89	1.90 TO 1.94	1.95 TO 1.99	2.00 TO 2.04	2.05 TO 2.09	2.10 TO 2.14	2.15 TO 2.19	2.20 TO 2.24	2.25 TO 2.29	2.30 TO 2.34	2.35 TO 2.39	2.40 TO 2.44	2.45 TO 2.49	2.50 TO 2.54
1.	1					1													
2.									1	1	1			1					
3.						1	1												
4.						5	2	3	3						1				
5.				2	4	5	8	1	3	1									
6.													1						
7.							1		1										
8.					1			1			1						1		
9.						1	1	11	10	10	6	5	1	2		1	2		
10.							1												
11.						1		1											
12.					2		4	2	7	3	4	2	3						
13.						1		3	1	2	1								
14.				2	3	12	21	12	6	6	5								
15.						3	6	4	3	1									
16.				1	2	2	3	5	3	1	1	1	1						
17.									3	2	1	1							
18.						2	4		1	1	1								
19.								1	2	1				1					
20.		1		1	2	7	22	42	43	38	31	10	9	2	1				
21.							1	3	2	1									
22.							2	9	12	3	7	4	5	2	1				
23.						1		4	7	5	8	3	1						
24.							1		1										
25.			1		2	15	26	43	50	57	42	17	12	2	1	2	1		
26.								2	1	1		2							
27.					1		1	6	6	17	18	22	16	5	5	1	1		1
28.					1	1	2	5	13	8	7	3	3	1	2	1			
29.								2	3	3	3	8	1	5					
	1	1	1	6	18	58	107	160	182	167	137	78	53	21	11	5	5		1

standard deviation and coefficient of variation have been calculated for most of the series, and will be placed on file at the SMITHSONIAN INSTITUTION, and the ROYAL SOCIETY OF EDINBURGH.

LYMNAEA PALUSTRIS

LENGTH / APERTURE LENGTH

SERIES NO.	1.55 TO 1.59	1.60 TO 1.64	1.65 TO 1.69	1.70 TO 1.74	1.75 TO 1.79	1.80 TO 1.84	1.85 TO 1.89	1.90 TO 1.94	1.95 TO 1.99	2.00 TO 2.04	2.05 TO 2.09	2.10 TO 2.14	2.15 TO 2.19	2.20 TO 2.24	2.25 TO 2.29	2.30 TO 2.34	2.35 TO 2.39	2.40 TO 2.44	2.45 TO 2.49	2.50 TO 2.54	2.55 TO 2.59
1.							1	1													
2.													2		1				1		
3.						1							1								
4.				2	1	4	5	1					1								
5.							3	5	7	6	2			1							
6.										1											
7.						1			1												
8.								1		1				2							
9.			1			1	8	7	10	6	9	4	1	1	1		1				
10.						1															
11.							1		1												
12.					1	2	7	3	5	4	4	1									
13.					1		2	2		2	1										
14.	1	3	5	15	16	9	10	6	1		1										
15.					2	4	4	3	3	1											
16.		1	2		2	4	4	3	2		1		1								
17.									1	1	2	1			1		1				
18.					1	1	1	4				2									
19.												1	1	1	1			1			
20.				1	1	2	5	17	34	42	28	39	24	9	1	5		1			
21.						1	1		3	1	1										
22.							2	6	8	10	11	7	2	2	2						
23.				1	1	2	3	2	9	8	1	1	1								
24.									1		1										
25.				1	2	9	19	44	45	54	38	33	13	4	4		2	1	1		1
26.							2		2	1			1								
27.						1		6	6	7	15	19	15	16	10	3	1	1			
28.						2	2	8	9	9	9	4		1	1	1	1				
29.							2	5	4	2	4	5		3							
	1	4	8	20	28	45	82	124	150	157	129	117	62	41	22	9	6	4	2		1

LYMNAEA PALUSTRIS

SERIES NO.	APERTURE LENGTH / APERTURE WIDTH																		
	1.20 TO 1.24	1.25 TO 1.29	1.30 TO 1.34	1.35 TO 1.39	1.40 TO 1.44	1.45 TO 1.49	1.50 TO 1.54	1.55 TO 1.59	1.60 TO 1.64	1.65 TO 1.69	1.70 TO 1.74	1.75 TO 1.79	1.80 TO 1.84	1.85 TO 1.89	1.90 TO 1.94	1.95 TO 1.99	2.00 TO 2.04	2.05 TO 2.09	2.10 TO 2.14
1.							1	1											
2.							2	1			1								
3.				1			1												
4.								2	2	3	5	2							
5.						2	6	8	4	2	1				1				
6.									1										
7.						1			1										
8.				1			1		1		1								
9.							7	4	10	7	11	3	6	2					
10.								1											
11.								1		1									
12.							1	2	3	7	9	3	1	1					
13.							1	4				1	2						
14.							1		4	7	14	14	9	9	3	2	2		2
15.					1	2	3	4	2	3	2								
16.							4	2	2	4	6	2							
17.						2	3				1	1							
18.						1	1	3	2	1	1								
19.				1	1	1	1	1											
20.				1	5	13	19	37	63	37	22	7	2	2	1				
21.							1		1		1	1	2	1					
22.					1		2	9	5	10	13	7		3					
23.							2	2	7	6	7	2	3						
24.							1	1											
25.	1		1	1	1	5	19	41	60	56	44	23	10	5	2	1	1		
26.				1				2	3										
27.							6	6	16	18	23	20	7	1	2		1		
28.						1	3	9	6	10	8	9	.	1					
29.							2	3	1	10	2	1	5	1					
	1		1	6	9	28	88	144	194	182	172	96	47	26	9	3	4		2

LYMNAEA PALUSTRIS

GREATER DIAMETER / APERTURE WIDTH

SERIES NO.	1.25 to 1.29	1.30 to 1.34	1.35 to 1.39	1.40 to 1.44	1.45 to 1.49	1.50 to 1.54	1.55 to 1.59	1.60 to 1.64	1.65 to 1.69	1.70 to 1.74	1.75 to 1.79	1.80 to 1.84	1.85 to 1.89	1.90 to 1.94	1.95 to 1.99	2.00 to 2.04	2.05 to 2.09	2.10 to 2.14
1.							1				1							
2.							2			1			1					
3.					1		1											
4.			1		1	1	4	5	2									
5.					1	1	5	8	3	4	1							1
6.					1													
7.				1		1												
8.			1				1		1	1								
9.			1	1	6	4	8	15	7	3	1	3	1					
10.						1												
11.							1		1									
12.						4	10	5	4	4								
13.					1	4		2		1								
14.			1		1	6	9	21	14	11	3	1						
15.				2	5	3	4	1	1	1								
16.			1	1			4	8		1								
17.					1	1	1	2	1		1							
18.					1	3	3	1		1								
19.					1	1		2			1							
20.					5	16	29	62	61	22	11	3						
21.							1	1	1	3	1							
22.			1			5	5	14	11	7	4	2	1					
23.			1		2	4	11	4	4	2	1							
24.					1		1											
25.	1			1	7	27	59	63	68	30	6	6	2	1				
26.					1	3	1	1										
27.			1		2	2	8	15	21	24	17	6	3	1				
28.				2	2	1	13	10	13	4	2							
29.					2	3	5	8	5	2								
	1		8	8	42	96	187	248	218	122	50	21	8	2				1

LYMNAEA EMARGINATA

LENGTH / GREATER DIAMETER

SERIES NO.	1.20 TO 1.24	1.25 TO 1.29	1.30 TO 1.34	1.35 TO 1.39	1.40 TO 1.44	1.45 TO 1.49	1.50 TO 1.54	1.55 TO 1.59	1.60 TO 1.64	1.65 TO 1.69	1.70 TO 1.74	1.75 TO 1.79	1.80 TO 1.84	1.85 TO 1.89	1.90 TO 1.94	1.95 TO 1.99	2.00 TO 2.04	2.05 TO 2.09	2.10 TO 2.14	2.15 TO 2.19
1.						2	5	4	5											
2.					2	3	2	1												
3.						1	1	3	6	3	10	5	2							
4.						1	2	2	1											
5.												4	3	4	1					
6.						1	11	14	10	3	1									
7.							2	3	1											
8.			2	1	1	3	5													
9.								1	1											
10.									3	3		1								
11.						1	2	2		1										
12.							1				1			1						
13.								4	10	5	11	4	1							
14.	1	1		2			1													
15.						1		1	5	6	4	2	1							
16.																2	1			
17.								1	2	4	21	25	23	11	8	4	1			
18.					2	2		1												
19.											2		2	1						
20.											1			2	2	2	2			1
21.						5	3	21	23	21	10	6	1							
22.											1	1			3			1		
23.		2	1	1																
24.				2	2	1	3	3	2											
25.				2	1															
26.	1		1	1	1															
	2	3	4	9	9	21	38	61	69	46	62	48	33	19	14	8	4	1		1

LYMNAEA EMARGINATA

LENGTH / APERTURE LENGTH

SERIES NO.	1.25 to 1.29	1.30 to 1.34	1.35 to 1.39	1.40 to 1.44	1.45 to 1.49	1.50 to 1.54	1.55 to 1.59	1.60 to 1.64	1.65 to 1.69	1.70 to 1.74	1.75 to 1.79	1.80 to 1.84	1.85 to 1.89	1.90 to 1.94	1.95 to 1.99	2.00 to 2.04	2.05 to 2.09	2.10 to 2.14	2.15 to 2.19
1.							4	4	4	2	2								
2.						2	4	1		1									
3.							1	4	6	8	7	5							
4.									2	1	2		1						
5.										2	2	2	5	1					
6.			2	4	12	16	4	1		1									
7.				1	1		2		2										
8.			3	3	4	1	1												
9.							2												
10.									1	2	4								
11.						3		1	1			1							
12.									1		1	1							
13.					2	2	9	16	5	1									
14.		1	2	1	1														
15.					1		2		2	3	4	4	1	3					
16.															2				1
17.								4	7	16	19	28	13	5	8				
18.				1		3	1												
19.								1	1	1	2								
20.										4		1	2	1	1		1		
21.					1	6	10	19	17	21	10	4	1		1				
22.									1		1	1			1			2	
23.		1	2					1											
24.					1	2	1	3	3	2	1								
25.		2				1													
26.	2		1		1														
	2	4	10	10	24	36	41	55	53	65	55	47	23	10	13		1	2	1

LYMNAEA EMARGINATA

SERIES NO.	APERTURE LENGTH / APERTURE WIDTH															
	1.10 to 1.14	1.15 to 1.19	1.20 to 1.24	1.25 to 1.29	1.30 to 1.34	1.35 to 1.39	1.40 to 1.44	1.45 to 1.49	1.50 to 1.54	1.55 to 1.59	1.60 to 1.64	1.65 to 1.69	1.70 to 1.74	1.75 to 1.79	1.80 to 1.84	1.85 to 1.89
1.				1		2	3	8		2						
2.						1	1	3	2	1						
3.								1		6	7	6	9	1	1	
4.	1	1	1		2	1										
5.						1		3	1	5		1		1		
6.					1	2	4	11	17	4						1
7.							1	1		3		1				
8.					2	3		2	1	3	1					
9.								1	1							
10.						3	1	1	1	1						
11.						1	1	4								
12.							2	1								
13.						1	5	5	8	10	3	1	2			
14.						1	1			2		1				
15.					1	1	1	5	4	2	3	2	1			
16.							2	1								
17.						2	12	29	27	20	9			1		
18.				2		1	1			1						
19.							1	1		2			1			
20.								1	2	1			2	2	2	
21.					2	20	18	24	12	9	5					
22.				1			1	2	1		1					
23.				1			2				1					
24.					3	3	2	2	2			1				
25.								1		1		1				
26.				1			1				1	1				
	1	1	1	6	11	43	60	107	79	73	31	15	15	5	3	1

LYMNAEA EMARGINATA

GREATER DIAMETER / APERTURE WIDTH

SERIES NO.	1.25 TO 1.29	1.30 TO 1.34	1.35 TO 1.39	1.40 TO 1.44	1.45 TO 1.49	1.50 TO 1.54	1.55 TO 1.59	1.60 TO 1.64	1.65 TO 1.69	1.70 TO 1.74	1.75 TO 1.79	1.80 TO 1.84	1.85 TO 1.89	1.90 TO 1.94
1.			1	1	2	4	6	2						
2.						2	5		1					
3.						1	5	5	6	7	4	1	1	1
4.	1		1	2	1		1							
5.				2	1	3	1	4	1					
6.		2	11	17	8		1				1			
7.					1	2	2		1					
8.		3	1	1	5	1	1							
9.						2								
10.				1	3	2	1							
11.				2	2	2								
12.			1			1	1							
13.	1	1	4	2	9	11	7							
14.						3	1			1				
15.						4	4	7	4		1			
16.			1		1		1							
17.			4	4	37	29	18	5	2	1				
18.			1	2	1		1							
19.		1			2	1		1						
20.					1	1	5	3						
21.		1	7	13	23	25	16	5						
22.				2	1	2	1							
23.						2	2							
24.					2	3	3	1	4					
25.				1			1	1						
26.					1	2				1				
	2	8	32	50	101	103	84	34	19	10	6	1	1	1

COMPARISON OF THE VARIATION OF THE TWO SPECIES

A study of the preceding data indicates with a considerable degree of certainty that there is an important difference in the type of variation exhibited by the two species investigated. In *Lymnaea palustris* the range of variation in any one locality tends to approximate that which occurs over the whole of the territory occupied by this animal. This is particularly remarkable in view of the fact that the collections of this species came from a wide variety of habitats. Series Nos. 25 and 27 came from ponds; Nos. 12 and 15 from intermittent streams; Nos. 16 and 20 from fresh water lakes; and No. 9 from a saline lake in which the concentration of mineral salts was 9688 parts per million (MOZLEY 1930). It is also interesting to observe that the same general principle regarding the relationship of local to geographical variation holds for all four ratios. It is particularly to be noted that this relationship holds true only in the particular region in which this work was done, and is known not to hold in other regions. In contrast to this, in *L. emarginata* the range of variation in any one locality forms only a part of the geographical variation.

The explanation of this diversity is believed to lie in the differences in the local distribution of the two species. Within the territory included in the scope of this paper, *L. palustris* occurs in a high proportion of the very numerous ponds and small lakes; for example, it was found in 238 of a series of 315 examined. These ponds and small lakes are often situated close together, and it is easy to understand that there are frequent opportunities for the mixture of stock. Under these conditions the appearance of recessive characters would be sporadic, the variation in all localities would be expected to be very similar, and its range wide.

In contrast to this continuous type of variation, is that of *L. emarginata*, which is found for the most part only on the rocky or bouldery shores of large lakes, a type of habitat which is of relatively rare occurrence in many parts of the country, sometimes being situated one or two hundred kilometers apart. There is thus little opportunity for genetic intermixture between the members of different colonies. Under these conditions recessive characters might be expected to appear in the bulk of the population in certain localities, and thus to form the basis of local races.

These facts may quite conceivably form the basis upon which many other natural experiments in isolation have taken place, and give some insight into a mechanism by means of which endemic faunas may have arisen.

It seems reasonable to suppose that this diversity is a matter of multiple factor inheritance. Many of the morphological characters which form the basis of the local races are in all probability recessive, and are masked,

or at least not much in evidence under conditions of free interbreeding, as occurs in *L. palustris* in this region. In *L. emarginata* the role of isolation appears to be connected with the segregation into colonies of small groups of individuals, in many cases the progeny of a single immigrant individual. Among these the selective action of the environment sometimes results in the predominance of a recessive character. The results of this work are therefore in accordance with those of SUMNER'S extensive investigations (1932) of the geographical races of deer mice. His results seem to lead to the conclusion that the extension of the Mendelian principle to include the inheritance of characters governed by multiple genetic factors is an adequate explanation of the transmission of subspecific differences. The present work may possibly give some further insight into the mechanism of a process by which such varieties may arise.

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THE GENETICS OF MAMMARY TUMOR INCIDENCE IN MICE*

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There have been a number of studies on the genetics of spontaneous tumors of the breast in mice. The chief weakness which they show, in common, is a lack of sufficient knowledge of the genetic composition of the foundation stocks used in respect to the incidence of mammary tumors. This results in inaccuracy in interpreting results because of unknown variables introduced by the parent strains.

With this in mind, the writers have attempted no outcrossing experiments between tumor and non-tumor strains until a sufficient period had elapsed and a large enough number of animals had been raised within a strain, under a system of close inbreeding (brother to sister or parent to offspring) to insure, as far as possible, genetic uniformity and reliability of the parent stocks used.

When a degree of inbreeding had been reached at which the material was sufficiently homogeneous to give reliable results out-crosses were made.

Because tumors of the mammary gland represent uncontrolled growth and because such a phenomenon is a very fundamental one as compared with many of the well-known mendelizing characters of rodents, reciprocal F_1 generations and F_2 generations descended from them were raised.

The present communication records and discusses the observed incidence of mammary tumors in the parent strains and in the reciprocal hybrid generations, and attempts to give a genetic interpretation to the experimental result.

MATERIALS AND METHODS

Parent Stock 1. Dilute brown dba (MURRAY-LITTLE)

The mammary tumor incidence in breeding females of this stock has been reported on in considerable detail by MURRAY (1934). While collecting these data it became evident to him that pregnancy and lactation influenced the rate and time of mammary tumor incidence. In the present experiment, therefore, virgin females carefully isolated from males at weaning (21–28 days of age) were used in the hybrid generations.

The incidence of mammary tumors in 297 virgin females of the dilute brown parent stock is shown in table 1. The animals are listed in successive thirty-day periods according to age of the individuals at the appearance of tumor, or its natural death without tumor.

* Part of the cost of the accompanying tables is paid by the GALTON AND MENDEL MEMORIAL FUND.

TABLE 1

Dilute brown virgin females (dba).

AGE IN DAYS	TUMOR MICE	PERCENT IN EACH AGE-GROUP	PERCENT DEAD AT EACH AGE	NON-TUMOR MICE	PERCENT IN EACH AGE-GROUP	PERCENT DEAD AT EACH AGE
241–270	1	0.66	0.66	0	0.00	0.00
271–300	2	1.32	1.98	1	0.68	0.68
301–330	0	0.00	1.98	3	2.05	2.73
331–360	4	2.64	4.62	9	6.16	8.89
361–390	12	7.94	12.56	20	13.69	22.58
391–420	19	12.58	25.14	12	8.21	30.79
421–450	23	15.23	40.37	16	10.95	41.74
451–480	12	7.94	48.31	12	8.21	49.95
481–510	18	11.92	60.23	12	8.21	58.16
511–540	6	3.97	64.20	14	9.58	67.74
541–570	14	9.27	73.47	17	11.64	79.38
571–600	15	9.93	83.40	4	2.73	82.11
601–630	5	3.31	86.71	2	1.36	83.47
631–660	8	5.29	92.00	4	2.73	86.20
661–690	4	2.64	94.64	5	3.42	89.62
691–720	2	1.32	95.96	8	5.47	95.09
721–750	5	3.31	99.27	4	2.73	97.82
751–780	1	0.66	100.00	3	2.05	100.00
	151			146		

It will be noted that the first of the 151 tumor mice occured in the 241–270 day age-period. The oldest occured in the 751–780 day age-group. In addition to the actual number of animals in each group the percent of the tumor or non-tumor animals falling within each successive age class is also shown.

The cumulative total of these percentages up to and including each successive age-group is given in the third and sixth lines of the table. Both populations naturally reach 100.0 percent as the last age-group is included. The tables of this type which are given for the *dba* virgin mice and for each hybrid generation are used as a basis for calculating the relative degree to which each generation fulfils the potential maximum of tumor formation. It is theoretically possible that a given generation of mice should give 100 percent tumor incidence, zero percent tumor incidence or some value lying between these extremes. The object is to devise some scale by which the different generations may be compared. Age and tumor incidence are the two main factors to be considered within any group of controlled genetic constitution [that is, a single generation]. The first principle that can safely be accepted is that the age at which the first tumor appears in a given generation can properly be taken as the lower age limit in our calculation. The percentage of all tumors of that generation which occur within the first age-group represents the chance which animals

living to the end of that age-group had of forming tumors. This value may be multiplied by the percent of all animals dying without tumor which falls within the age-group in question. This will give a figure which represents the unfulfilled chance of tumor formation expressed by the animals of that age-group. If this value is calculated for each succeeding age-group and the totals are added, a measure of the opportunities for tumor formation which were unfulfilled is obtained. If, for example, all non-tumor mice in a given generation survived beyond the age-group at which the last tumor of that generation appeared, the figure obtained would be 100.00 $\times$100.00 or 10,000. This expressed in its simplest form would be unity. On the other hand, if all non-tumor mice died before the formation of the first tumor the value obtained would be 100.00$\times$0.00 or 0.00. The actual figures lie in between and can be expressed as a decimal. For the dilute brown virgin females the value is .4842. This procedure has been followed for the various hybrid generations and will be referred to later.

The choice of a single value to represent the tumor incidence of a given strain is admittedly arbitrary. Actually the appearance of mammary tumors is, to a considerable degree, influenced by age and the physiological changes incidental to reproduction. As before stated, an attempt has been made to reduce the latter variable to as low a point as possible by using virgin females as material. Age, as a factor, will be examined in considerable detail.

TABLE 2

Dilute brown virgin females (dba).

AGE GROUP	TUMOR	DEATHS NON-TUMOR	NO. ALIVE AT START OF PERIOD	NO. WHICH LATER FORMED TUMORS	PERCENTAGE WHICH FORMED TUMORS
241–270	1	0	297	151	50.84
271–300	2	1	296	150	50.67
301–330	0	3	293	148	50.51
331–360	4	9	290	148	51.03
361–390	12	20	277	144	51.98
391–420	19	12	245	132	53.87
421–450	23	16	214	113	52.80
451–480	12	12	175	90	51.42
481–510	18	12	151	78	51.65
511–540	6	14	121	60	49.58
541–570	14	17	101	54	53.46
571–600	15	4	70	40	57.14
601–630	5	2	51	25	49.01
631–660	8	4	44	20	45.45
661–690	4	5	32	12	37.50
691–720	2	8	23	8	34.78
721–750	5	4	13	6	46.15
751–780	1	3	4	1	25.00

In addition to tabulating tumor incidence in any stock, as has been done in table 1, it is also possible to get an idea of the rate of tumor production at successive age periods by another method of tabulation of the same data as shown in table 2.

This method has already been described and used by MURRAY (l.c.). For any age-class, the number of animals dying of tumor and without

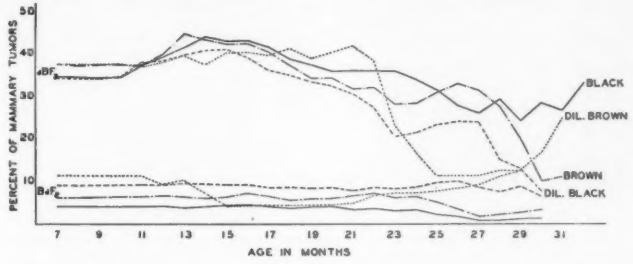

FIGURE 1.

tumor are recorded in separate columns. The total number of mice alive at the beginning of each age-period is shown in a third column. Those which during that period or later developed mammary tumors are listed in the fourth column. The tumor and non-tumor mice dying in each age-period are subtracted from the total and a percentage of tumor incidence for the whole remaining period, minus those age-groups already passed, is

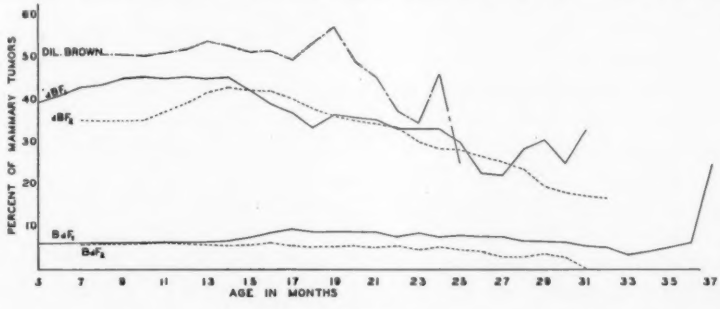

FIGURE 2.

calculated for each thirty day age-class. Any marked change in the rate of tumor incidence will be reflected in a rise or fall of the percentage. If the percentage is relatively constant in a number of consecutive age-groups, it denotes that the factors which are causing the formation of tumors are working at a steady rate.

The relative constancy of tumor incidence in virgin females in those age-

groups where a sufficient number of mice survive to make the data critical is very striking. The curve is very different from that shown by breeding females of the same stock (see MURRAY l.c). It is therefore evident that keeping the animals as virgins is an aid in simplifying the experiment.

If one wishes to give a single value to the tumor incidence of the strain the most logical thing to do is to include, in making the calculation, all animals that lived up to and beyond the age at which the first tumor appeared. When this is done for the dilute brown virgin mice the incidence of mammary tumors is 50.84 percent.

Parent Stock 2. C57 black

Since, in this stock, no malignant mammary tumors have been recorded since 1921 when inbreeding of the stock was started, there will of course, be only one group of animals, namely, "non-tumor." Since, also, it has been shown by several investigators that breeding females of any stock are more apt to form mammary tumors than virgins, it will be desirable to tabulate a distribution of age at death of 240 *breeding females* of this strain. Only those females have been included which survived to reach the age-group in which the earliest tumor has been recorded in virgin dilute browns. The others may be properly considered as having died before reaching the age at which any tendency to form tumor might be expected to express itself. This procedure really is deliberately weighing the data in favor of bringing out any possible tumor tendency that might be present in the C57 black strain. If they were compared with *breeding* dilute brown (*dba*) strain females, many C57 black animals, which died between the 91-120 day age-group at which the earliest tumor has been recorded in dilute brown breeders (MURRAY 1934) and the 241–270 age-group could be included. It seems desirable, however, to tabulate the data in a way which makes it as difficult as possible to establish C57 as a strain which does not form mammary tumors.

The age distribution of mice of the C57 black stock is shown in table 3. As in the previous table the percentage of mice dying in each age-group is given, as well as the cumulative death rate from period to period. It will be noted, by comparison with table 1, that 6.73 percent of the C57 black mice lived longer than the oldest animal in the dilute brown stock, thus giving the non-tumor mice ample time to form mammary tumors had they possessed the constitutional tendency to do so.

METHODS

In the reciprocal crosses made between these two strains of mice, animals were mated at weaning (that is, 28 days of age) in order to avoid the possibility of previous fertilization. In each case the animals chosen to

TABLE 3

C57 black breeding females (DBa).

AGE IN DAYS	NO. DYING	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE
241–270	18	7.50	7.50
271–300	28	11.66	19.16
301–330	23	9.58	28.74
331–360	18	7.50	36.24
361–390	15	6.25	42.49
391–420	20	8.33	50.82
421–450	6	2.50	53.32
451–480	14	5.83	59.15
481–510	6	2.50	61.65
511–540	7	2.91	64.56
541–570	4	1.66	66.22
571–600	6	2.50	68.72
601–630	8	3.33	72.05
631–660	12	5.00	77.05
661–690	8	3.33	80.38
691–720	13	5.41	85.79
721–750	7	2.91	88.70
751–780	11	4.58	93.28
781–810	2	0.83	94.11
811–840	4	1.66	95.77
841–870	1	0.41	96.18
871–900	6	2.50	98.68
901–930	2	0.83	99.51
931–960	1	0.41	100.00
	240		

make the cross were born and raised in pens in which no adult males were present, thus precluding the chance of their being fertilized. The date at which they produced F_1 young formed an additional and conclusive test of their virginity at the time of mating.

The F_1 litters were divided. Part of the animals were mated at weaning to produce the F_2 generation while part were isolated as virgin females to serve as material from which the rate of tumor incidence in the F_1 generations could be calculated.

The females of the F_2 generation were saved as virgins to give data on the tumor incidence in that generation.

Every animal throughout the experiment was individually identified and recorded.

EXPERIMENTAL RESULTS

Reciprocal F_1 Generations

In the *dB* F_1 generation (dilute brown high tumor ♀ × C57 black non-tumor ♂) 113 females, saved as virgins, lived to an age sufficient to be included in the experiment. Of these 45, or 39.82 percent, developed

mammary tumors. All mice of this generation were black in color, being phenotypically indistinguishable from the C57 stock mice. The age distribution of tumor and non-tumor mice of this generation is given in table 4.

TABLE 4

dB F_1 generation.

AGE IN DAYS	TUMOR MICE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	NON-TUMOR MICE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE
151–180	1	2.22	2.22	5	7.35	7.35
181–210	0	0.00	2.22	5	7.35	14.70
211–240	1	2.22	4.44	3	4.41	19.11
241–270	0	0.00	4.44	3	4.41	23.52
271–300	0	0.00	4.44	1	1.47	24.99
301–330	1	2.22	6.66	0	0.00	24.99
331–360	0	0.00	6.66	1	1.47	26.46
361–390	1	2.22	8.88	0	0.00	26.46
391–420	1	2.22	11.10	2	2.94	29.40
421–450	5	11.11	22.21	1	1.47	30.87
451–480	5	11.11	33.32	1	1.47	32.34
481–510	4	8.88	42.20	2	2.94	35.28
511–540	5	11.11	53.31	3	4.41	39.69
541–570	0	0.00	53.31	5	7.35	47.04
571–600	3	6.66	59.97	4	5.88	52.92
601–630	2	4.44	64.41	3	4.41	57.33
631–660	2	4.44	68.85	1	1.47	58.80
661–690	2	4.44	73.29	4	5.88	64.68
691–720	1	2.22	75.51	2	2.94	67.62
721–750	2	4.44	79.95	1	1.47	69.09
751–780	4	8.88	88.83	4	5.88	74.97
781–810	1	2.22	91.05	3	4.41	79.38
811–840	0	0.00	91.05	4	5.88	85.26
841–870	0	0.00	91.05	1	1.47	86.73
871–900	2	4.44	95.49	3	4.41	91.14
901–930	0	0.00	95.49	2	2.94	94.08
931–960	2	4.44	100.00	2	2.94	97.02
961–990	0	0.00		2	2.94	100.00
	45			68		

It will be noted that a considerable number of the animals produced mammary tumors and that they did so over a period which began earlier and continued longer than in the pure dilute brown stock (table 1).

The cumulative percentage of tumor incidence for successive age periods is given in table 4. So also is the cumulative percentages of the death rate of non-tumor mice. When a value to indicate the relative unfulfilled opportunity for tumor formation is calculated by the method described in the section on materials (*dba* stock) it is found to be .3941, where unity would signify that all non-tumor mice of this generation lived longer than the last mouse which formed a tumor.

The tabulation of the tumor incidence rate in this generation is given in table 5. The graphic representation of these data will be found in figure 2, which will be considered when a discussion of the results is attempted.

TABLE 5

dB F_1 *generation.*

AGE GROUP	DEATHS		NO. ALIVE AT START OF PERIOD	NO. WHICH LATER FORMED TUMORS	PERCENTAGE WHICH FORMED TUMORS
	TUMOR	NON-TUMOR			
151–180	1	5	113	45	39.82
181–210	0	5	107	44	41.12
211–240	1	3	102	44	43.13
241–270	0	3	98	43	43.87
271–300	0	1	95	43	45.26
301–330	1	0	94	43	45.74
331–360	0	1	93	42	45.16
361–390	1	0	92	42	45.65
391–420	1	2	91	41	45.05
421–450	5	1	88	40	45.45
451–480	5	1	82	35	42.68
481–510	4	2	76	30	39.47
511–540	5	3	70	26	37.14
541–570	0	5	62	21	33.87
571–600	3	4	57	21	36.84
601–630	2	3	50	18	36.00
631–660	2	1	45	16	35.55
661–690	2	4	42	14	33.33
691–720	1	2	36	12	33.33
721–750	2	1	33	11	33.33
751–780	4	4	30	9	30.00
781–810	1	3	22	5	22.72
811–840	0	4	18	4	22.22
841–870	0	1	14	4	28.57
871–900	2	3	13	4	30.76
901–930	0	2	8	2	25.00
931–960	2	2	6	2	33.00
961–990	0	2	2	0	

In the *Bd* F_1 generation (C57 black non-tumor ♀ × dilute brown high tumor ♂) the result was very different. Of the 356 females saved as virgins, only 23 or 6.05 percent developed mammary tumors. The age distribution is of interest. It is given in table 6.

The earliest age at which animals were included for tabulation is the same as that at which the earliest tumor appeared in the *dB* F_1 generation. This is logical from a genetic point of view since under random assortment and normal segregation of the chromosomes, the females of the two F_1 generations should be identical.

It will be noted that the *Bd* F_1 generation has 28 animals which lived

TABLE 6

Bd F_1 *generation.*

AGE IN DAYS	TUMOR MICE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	NON-TUMOR MICE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE
151– 180				1	0.28	0.28
181– 210				0	0.00	0.28
211– 240				3	0.84	1.12
241– 270				1	0.28	1.40
271– 300				9	2.52	3.92
301– 330				8	2.24	6.16
331– 360	1	4.34	4.34	13	3.65	9.81
361– 390	1	4.34	8.68	20	5.61	15.42
391– 420	1	4.34	13.02	30	8.42	23.84
421– 450	0	0.00	13.02	28	7.86	31.70
451– 480	0	0.00	13.02	29	8.14	39.84
481– 510	0	0.00	13.02	18	5.05	44.89
511– 540	2	8.69	21.71	8	2.24	47.13
541– 570	0	0.00	21.71	3	0.84	47.97
571– 600	1	4.34	26.05	9	2.52	50.49
601 - 630	1	4.34	30.39	9	2.52	53.01
631– 660	3	13.04	43.43	13	3.65	56.66
661– 690	0	0.00	43.43	13	3.65	60.31
691– 720	2	8.69	52.12	10	2.80	63.11
721– 750	1	4.34	56.46	16	4.49	67.60
751– 780	1	4.34	60.80	10	2.80	70.40
781– 810	1	4.34	65.14	11	3.08	73.48
811– 840	2	8.69	73.83	13	3.65	77.13
841– 870	1	4.34	78.17	12	3.37	80.50
871– 900	1	4.34	82.51	11	3.08	83.58
901– 930	1	4.34	86.85	6	1.68	85.26
931– 960	1	4.34	91.19	15	4.21	89.47
961– 990	1	4.34	95.53	10	2.80	92.27
991–1020	0	0.00	95.53	4	1.12	93.39
1021–1050	0	0.00	95.53	5	1.40	94.79
1051–1080	0	0.00	95.53	4	1.12	95.91
1081–1110	0	0.00	95.53	11	3.08	98.99
1111–1140	1	4.34	100.00	2	0.56	99.55
1141–1170				0	0.00	99.55
1171–1200				1	0.28	100.00
	23			356		

longer than the oldest mouse in the *dB* F_1 generation. Thirty-nine animals or 10.28 percent lived longer than any of the C57 blacks included in table 3. One hundred and fourteen or 30.08 percent lived longer than the oldest dilute brown included in table 1. This gave ample opportunity for animals of the *Bd* F_1 generation to form mammary tumors. The ages at which mice of this generation formed such tumors overlapped definitely the ages at which the dilute brown stock and the *dB* F_1 mice did so. Thus 91.3 percent of the *Bd* F_1 tumors fell within the range of age-groups in which the *dB* F_1

tumors appeared. Almost sixty-one percent overlapped with the ages at which dilute brown tumors were recorded.

For this generation as for its reciprocal F_1 (*dB* F_1) a value for the relative amount of unfulfilled opportunity for tumor formation has been calculated. The figure obtained is .4392.

The tumor incidence rate in the *Bd* F_1 generation is given in table 7. The comparison between this distribution and that of the *dB* F_1 and parent *dba* generations are shown graphically in figure 2 and will be considered further in the general discussion.

TABLE 7

Bd F_1 *generation.*

AGE GROUP	DEATHS		NO. ALIVE AT START OF PERIOD	NO. WHICH LATER FORMED TUMORS	PERCENTAGE WHICH FORMED TUMORS
	TUMOR	NON-TUMOR			
151– 180	0	1	379	23	6.06
181– 210	0	0	378	23	6.08
211– 240	0	3	378	23	6.08
241– 270	0	1	375	23	6.13
271– 300	0	9	374	23	6.14
301– 330	0	8	365	23	6.30
331– 360	1	13	357	23	6.44
361– 390	1	20	343	22	6.41
391– 420	1	30	322	21	6.52
421– 450	0	28	291	20	6.87
451– 480	0	29	263	20	7.60
481– 510	0	18	234	20	8.54
511– 540	2	8	216	20	9.25
541– 570	0	3	206	18	8.73
571– 600	1	9	203	18	8.86
601– 630	1	9	193	17	8.80
631– 660	3	13	183	16	8.74
661– 690	0	13	167	13	7.78
691– 720	2	10	154	13	8.44
721– 750	1	16	142	11	7.74
751– 780	1	10	125	10	8.00
781– 810	1	11	114	9	7.89
811– 840	2	13	102	8	7.84
841– 870	1	12	87	6	6.89
871– 900	1	11	74	5	6.75
901– 930	1	6	62	4	6.45
931– 960	1	15	55	3	5.45
961– 990	1	10	39	2	5.12
991–1020	0	4	28	1	3.57
1021–1050	0	5	24	1	4.16
1051–1080	0	4	19	1	5.26
1081–1110	0	11	15	1	6.66
1111–1140	1	2	4	1	25.00
1141–1170	0	0	1	0	
1171–1200	0	1	1	0	

F_2 GENERATIONS

The *dB* F_2 generation was produced by mating brother and sister *dB* F_1 animals. It consisted of 664 virgin females which lived to an age equal or superior to that at which the first mammary tumor of the F_2 generation appeared.

In this generation a di-hybrid ratio of coat colors due to the segregation of B-black and b-brown and D-intense, d-"blue" dilution is expected as follows:

	BLACK	DILUTE BLACK	BROWN	DILUTE BROWN	TOTAL
Expected	373.5	124.5	124.5	41.5	664
Observed	374	116	137	37	664

The observed figures obviously show random recombination of the two pairs of genes.

When the variously colored animals of this generation are classified according to whether or not they have mammary tumors the following results are obtained:

COLOR	NON-TUMOR	TUMOR	PERCENT TUMOR
Black	244	130	34.75
Dilute black	76	40	34.48
Brown	85	52	37.95
Dilute brown	23	14	37.83
Total	428	236	35.54

When the two pairs of genes are tabulated separately the figures are as follows:

COLOR	NON-TUMOR	TUMOR	PERCENT TUMOR
All blacks (B)	320	170	34.69
All browns (b)	108	66	37.93
All intense (D)	329	182	35.61
All dilute (d)	99	54	35.29

The age distributions of the various color classes are shown in table 8.

It should be noted that the first mammary tumor in this generation appears in the 211–240 day age-group as compared with the earliest tumor in F_1 which was in the 151-180 day age-group. Classes before the 211-240 day age-group have been omitted from the F_2 tables. This procedure is based on the following facts: The two F_1 generations being presumably genetically similar, can be fairly compared with one another but cannot be taken as a standard for the F_2 generations. These, being comparable with one another, must form their own standard. These data are reduced to a percentage basis in table 9. Two types of distribution are shown.

On the upper line of each color class is shown the percentage of animals of that color which had cancer or died in each succeeding age-group. On the lower line of each color class is shown the percent of all animals of that

TABLE 8

dB F_2 generation.

AGE IN DAYS	NON-TUMOR MICE					TUMOR MICE				
	BLACK	DILUTE BLACK	BROWN	DILUTE BROWN	TOTAL	BLACK	DILUTE BLACK	BROWN	DILUTE BROWN	TOTAL
211– 240	0	0	0	0	0	0	0	1	0	1
241– 270	0	0	1	0	1	1	0	0	0	1
271– 300	3	1	0	0	4	1	0	0	0	1
301– 330	33	12	6	1	52	4	1	4	1	10
331– 360	29	6	11	3	49	7	3	2	1	13
361– 390	32	3	17	1	53	11	0	4	0	15
391– 420	25	4	5	0	34	9	1	6	1	17
421– 450	10	2	2	2	16	12	1	3	0	16
451– 480	5	2	3	0	10	3	4	2	0	9
481– 510	3	1	1	1	6	7	4	3	1	15
511– 540	4	1	1	1	7	11	2	4	0	17
541– 570	5	3	0	0	8	6	3	3	1	13
571– 600	10	2	2	1	15	10	2	1	0	13
601– 630	13	3	2	2	20	7	3	3	1	14
631– 660	9	2	5	0	16	5	3	2	1	11
661– 690	5	3	1	1	10	3	5	3	4	15
691– 720	6	2	3	0	11	6	0	1	1	8
721– 750	7	3	3	2	15	6	0	0	1	7
751– 780	9	4	6	0	19	7	1	2	0	10
781– 810	5	3	1	0	9	3	1	1	0	5
811– 840	7	2	2	1	12	1	3	2	0	6
841– 870	2	4	1	0	7	3	1	2	0	6
871– 900	7	1	3	2	13	1	1	2	0	4
901– 930	4	5	1	2	12	2	1	0	0	3
931– 960	5	5	3	1	14	1	0	1	1	3
961– 990	4	2	1	1	8	3	0	0	0	3
991–1020	2	0	4	1	7	0	0	0	0	0
	244	76	85	23	428	130	40	52	14	236

color that have died up to and including each successive age-group. This succession of figure is, of course, cumulative for any given color class and reaches 100 percent at the last age-group in which any mice of that color and generation appear.

It is interesting to calculate the relative unfulfilled opportunity to form tumors which the four color classes of this generation have shown. The values obtained are as follows:

Black	.3887
Dilute black	.4842
Brown	.4552
Dilute brown	.5272

Thus the dilute classes have had actually a greater opportunity to form tumors than have the intense classes. Figures already given, however,

TABLE 9

dB F_2 generation.

AGE IN DAYS	BLACK NON-TUMOR		DILUTE BLACK NON-TUMOR		BROWN NON-TUMOR		DILUTE BROWN NON-TUMOR		TOTAL NON-TUMOR		BLACK TUMOR		DILUTE BLACK TUMOR		BROWN TUMOR		DILUTE BROWN TUMOR		TOTAL TUMOR	
	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE
211– 240	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.92	1.92	0.00	0.00	0.42	0.42
241– 270	0.00	0.00	0.00	0.00	1.17	1.17	0.00	0.00	0.23	0.23	0.76	0.76	0.00	0.00	0.00	1.92	0.00	0.00	0.42	0.84
271– 300	1.22	1.22	1.31	1.31	0.00	1.17	0.00	0.00	0.93	1.16	0.76	1.52	0.00	0.00	0.00	1.92	0.00	0.00	0.42	1.26
301– 330	13.52	14.74	15.78	17.09	7.05	8.22	4.34	4.34	12.14	13.30	3.07	4.59	2.50	2.50	7.69	9.61	7.14	7.14	4.23	5.49
331– 360	11.88	26.62	7.89	24.98	12.94	21.16	13.04	17.38	11.44	24.74	5.38	9.97	7.50	10.00	3.84	13.45	7.14	14.28	5.50	10.99
361– 390	13.11	39.73	3.94	28.92	20.00	41.16	4.34	21.72	12.38	37.12	8.46	18.43	0.00	10.00	7.69	21.14	0.00	14.28	6.35	17.34
391– 420	10.24	49.97	5.26	34.18	5.88	47.04	0.00	21.72	7.94	45.06	6.92	25.35	2.50	12.50	11.53	32.67	7.14	21.42	7.20	24.54
421– 450	4.09	54.06	2.63	36.81	2.35	49.39	8.69	30.41	3.73	48.79	9.23	34.58	2.50	15.00	5.76	38.43	0.00	21.42	6.77	31.31
451– 480	2.04	56.10	2.63	39.44	3.52	52.91	0.00	30.41	2.33	51.12	5.38	39.96	10.00	25.00	3.84	42.27	0.00	21.42	3.81	35.12
481– 510	1.22	57.32	1.31	40.75	1.17	54.08	4.34	34.75	1.40	52.52	2.30	42.36	10.00	35.00	5.76	48.03	7.14	28.56	6.35	41.47
511– 540	1.63	58.95	1.31	42.06	1.17	55.25	4.34	39.09	1.63	54.15	8.46	50.72	5.00	40.00	7.69	55.72	0.00	28.56	7.20	48.67
541– 570	2.04	60.99	3.94	46.00	0.00	55.25	0.00	39.09	1.86	56.01	4.61	55.33	7.50	47.50	5.76	61.48	7.14	35.70	5.50	54.17
571– 600	4.09	65.08	2.63	48.63	2.35	57.60	4.34	43.43	3.50	59.51	7.69	63.02	5.00	52.50	1.92	63.40	0.00	35.70	5.50	59.67
601– 630	5.32	70.40	3.94	52.57	2.35	59.95	8.69	52.12	4.67	64.18	5.38	68.40	7.50	60.00	5.76	69.16	7.14	42.84	5.93	65.60
631– 660	3.68	74.08	2.63	55.20	5.88	65.83	0.00	52.12	3.73	67.91	3.84	72.24	7.50	67.50	3.84	73.00	7.14	49.98	4.66	70.26
661– 690	2.04	76.12	3.94	59.14	1.17	67.00	4.34	56.46	2.33	70.24	2.30	74.54	12.50	80.00	5.76	78.76	28.57	78.55	6.35	76.61
691– 720	2.45	78.57	2.63	61.77	3.52	70.52	0.00	56.46	2.57	72.81	4.61	79.15	0.00	80.00	1.92	80.68	7.14	85.69	3.38	79.99
721– 750	2.86	81.43	3.94	65.71	3.52	74.04	8.69	65.15	3.50	76.31	4.61	83.76	0.00	80.00	0.00	80.68	7.14	92.83	2.96	82.95
751– 780	3.68	85.11	5.26	70.97	7.05	81.09	0.00	65.15	4.43	80.74	5.38	89.14	2.50	82.50	3.84	84.52	0.00	92.83	4.23	87.18
781– 810	2.04	87.15	3.94	74.91	1.17	82.26	0.00	65.15	2.10	82.84	2.30	91.44	2.50	85.00	1.92	86.44	0.00	92.83	2.11	89.29
811– 840	2.86	90.01	2.63	77.54	2.35	84.61	4.34	69.49	2.80	85.64	0.76	92.20	7.50	92.50	3.84	90.28	0.00	92.83	2.54	91.83
841– 870	0.81	90.82	5.26	82.80	1.17	85.78	0.00	69.49	1.63	87.27	2.30	94.50	2.50	95.00	3.84	94.12	0.00	92.83	2.54	94.37
871– 900	2.86	93.68	1.31	84.11	3.52	89.30	8.69	78.18	3.03	90.30	0.76	95.26	2.50	97.50	3.84	97.96	0.00	92.83	1.69	96.06
901– 930	1.63	95.31	6.57	90.68	1.17	90.47	8.69	86.87	2.80	93.10	1.53	96.79	2.50	100.00	0.00	97.96	0.00	92.83	1.27	97.33
931– 960	2.04	97.35	6.57	97.25	3.52	93.99	4.34	91.21	3.27	96.37	0.76	97.55			1.92	100.00	7.14	100.00	1.27	98.60
961– 990	1.63	98.98	2.63	100.00	1.17	95.16	4.34	95.55	1.86	98.23	2.30	100.00							1.27	100.00
991–1020	0.81	100.00			4.70	100.00	4.34	100.00	1.63	100.00										

show that the tumor incidence in the two types were essentially the same, being 35.29 percent for dilute animals and 35.61 percent for intense mice.

As in the case of F_1 generations it has seemed desirable to tabulate the rate of tumor incidence for the F_2 mice also. The data for the *dB* F_2 animals are given in table 10.

TABLE 10

dB F_2 *generation.*

AGE GROUP	DEATHS		NO. ALIVE AT START OF PERIOD	NO. WHICH LATER FORMED TUMORS	PERCENTAGE WHICH FORMED TUMORS
	TUMOR	NON-TUMOR			
211–240	1	0	664	236	35.54
241–270	1	1	663	235	35.44
271–300	1	4	661	234	35.40
301–330	10	52	656	233	35.51
331–360	13	49	594	223	37.54
361–390	15	53	532	210	39.47
391–420	17	34	464	195	42.02
421–450	16	16	413	178	43.09
451–480	9	10	381	162	42.51
481–510	15	6	362	153	42.26
511–540	17	7	341	138	40.46
541–570	13	8	317	121	38.17
571–600	13	15	296	108	36.48
601–630	14	20	268	95	35.44
631–660	11	16	234	81	34.61
661–690	15	10	207	70	33.81
691–720	8	11	182	55	30.21
721–750	7	15	163	47	28.83
751–780	10	19	141	40	28.36
781–810	5	9	112	30	26.78
811–840	6	12	98	25	25.51
841–870	6	7	80	19	23.75
871–900	4	13	67	13	19.40
901–930	3	12	50	9	18.00
931–960	3	14	35	6	17.14
961–990	3	8	18	3	16.66
991–1020	0	7	7	0	

These data in the form of curves are further considered in figures 1 and 2 under general discussion of the relationship of the reciprocal crosses to one another.

The *Bd* F_2 generation formed from brother to sister matings of *Bd* F_1 animals consists of 687 virgin females which lived up to or beyond the age at which the first F_2 generation tumor appeared.

As in the *dB* F_2 generation four color classes are expected. The comparison of the observed figures with those calculated for a 9:3:3:1 ratio is as follows:

	BLACK	DILUTE BLACK	BROWN	DILUTE BROWN	TOTAL
Expected	387	129	129	43	688
Observed	379	132	141	35	687

The experimental results obviously coincide closely with the expectation and are indicative of segregation and random recombination.

When the four color classes are tabulated according to the incidence of mammary tumors the following results are obtained:

COLOR	NON-TUMOR	TUMOR	PERCENT TUMOR
Black	363	16	4.22
Dilute black	120	12	9.09
Brown	132	9	6.38
Dilute brown	31	4	11.42
Total	646	41	5.96

TABLE 11

Bd F_2 generation.

AGE IN DAYS	NON-TUMOR MICE					TUMOR MICE				
	BLACK	DILUTE BLACK	BROWN	DILUTE BROWN	TOTAL	BLACK	DILUTE BLACK	BROWN	DILUTE BROWN	TOTAL
211– 240	1	0	1	0	2	0	0	0	0	0
241– 270	1	0	0	0	1	0	0	0	0	0
271– 300	2	1	2	0	5	0	0	0	0	0
301– 330	9	3	1	0	13	0	0	0	0	0
331– 360	16	6	4	2	28	1	1	0	1	3
361– 390	14	7	7	3	31	2	0	1	0	3
391– 420	18	7	9	1	35	0	1	1	1	3
421– 450	28	7	7	3	45	0	1	0	1	2
451– 480	13	1	13	1	28	0	0	0	0	0
481– 510	7	3	1	0	11	2	1	1	0	4
511– 540	5	2	6	0	13	1	0	1	0	2
541– 570	14	6	3	0	23	0	1	0	0	1
571– 600	11	3	2	0	16	0	0	0	0	0
601– 630	8	4	6	2	20	2	1	0	0	3
631– 660	14	6	5	5	30	0	0	0	0	0
661– 690	13	7	5	1	26	2	1	1	0	4
691– 720	16	4	3	0	23	0	0	0	0	0
721– 750	10	7	4	1	22	2	0	1	0	3
751– 780	12	1	0	1	14	1	0	1	0	2
781– 810	20	3	3	1	27	2	1	1	0	4
811– 840	21	5	8	2	36	0	1	0	0	1
841– 870	20	6	7	1	34	0	0	0	0	0
871– 900	20	2	8	1	31	0	1	0	1	2
901– 930	20	10	6	1	37	1	2	1	0	4
931– 960	24	5	10	2	41	0	0	0	0	0
961– 990	13	8	6	1	28	0	0	0	0	0
991–1020	10	2	4	1	17	0	0	0	0	0
1021–1050	1	2	1	1	5	0	0	0	0	0
1051–1080	2	1	0	0	3	0	0	0	0	0
1081–1110	0	1	0	0	1	0	0	0	0	0
	363	120	132	31	646	16	12	9	4	41

The two pairs of genes considered separately give the following figures:

COLOR	NON-TUMOR	TUMOR	PERCENT TUMOR
All black (B)	483	28	5.47
All brown (b)	163	13	7.38
All intense (D)	495	25	4.80
All dilute (d)	151	16	9.58

The age distribution by colors and according to whether the animals are tumorous or not are given, for the *Bd* F_2 generation, in table 11.

These data are reduced to a percentage basis in table 12. Two types of distribution are shown as in the similar tables for the *dB* F_2 generation.

The data for the four color classes in the *Bd* F_2 generation can be analyzed, as were those for *dB* F_2 in respect to the relative unused opportunity for tumor formation. When this is done, the values are as follows:

Black	.5644
Dilute black	.4898
Brown	.5223
Dilute brown	.6606

The data showing the rate of tumor incidence for the *Bd* F_2 generation are given in table 13.

As before stated these data are presented graphically in figures 1 and 2. They will be discussed later.

COMPARISON OF *dB* AND *Bd* CROSSES

A most striking and important fact to bear in mind is the obvious and consistent difference in the incidence of mammary tumors in animals derived from the *dB* cross on the one hand and the *Bd* cross on the other hand. A preliminary note on the existence of a difference in reciprocal crosses between high and low tumor strains has been published (1933) by the staff of the ROSCOE B. JACKSON MEMORIAL LABORATORY. The present figures are the extension of Experiment A reported in the communication. It is also of interest to note that the difference in that incidence of tumors in the reciprocal crosses of these strains was independently observed and recorded by KORTEWEG (1934) who had for some years been working with mice descended from material sent him at Amsterdam.

The F_1 and F_2 generations may be tabulated for purposes of comparison, in a condensed form as follows:

GENERATION	NO. OF VIRGIN ♀s	NON-TUMOR	MAMMARY TUMOR	PERCENT MAMMARY TUMOR
dB F_1	113	68	45	39.82
dB F_2	664	428	236	35.54
Bd F_1	379	356	23	6.06
Bd F_2	687	646	41	5.96

TABLE 12

Bd F_2 generation.

AGE IN DAYS	BLACK NON-TUMOR		DILUTE BLACK NON-TUMOR		BROWN NON-TUMOR		DILUTE BROWN NON-TUMOR		TOTAL NON-TUMOR		BLACK TUMOR		DILUTE BLACK TUMOR		BROWN TUMOR		DILUTE BROWN TUMOR		TOTAL TUMOR	
	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE	PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE
211– 240	0.27	0.27	0.00	0.00	0.75	0.75	0.00	0.00	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
241– 270	0.27	0.54	0.00	0.00	0.00	0.75	0.00	0.00	0.15	0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
271– 300	0.55	1.09	0.83	0.83	1.51	2.26	0.00	0.00	0.77	1.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
301– 330	2.47	3.56	2.50	3.33	0.75	3.01	0.00	0.00	2.01	3.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
331– 360	4.40	7.96	5.00	8.33	3.03	6.04	6.45	6.45	4.33	7.56	6.25	6.25	8.33	8.33	0.00	0.00	25.00	25.00	7.31	7.31
361– 390	3.85	11.81	5.83	14.16	5.30	11.34	9.67	16.12	4.79	12.35	12.50	18.75	0.00	8.33	11.11	11.11	0.00	25.00	7.31	14.62
391– 420	4.95	16.76	5.83	19.99	6.81	18.15	3.22	19.34	5.41	17.76	0.00	18.75	8.33	16.66	11.11	22.22	25.00	50.00	7.31	21.93
421– 450	7.71	24.47	5.83	25.82	5.30	23.45	9.67	29.01	6.96	24.72	0.00	18.75	8.33	24.99	0.00	22.22	25.00	75.00	4.87	26.80
451– 480	3.58	28.05	0.83	26.65	9.84	33.29	3.22	32.23	4.33	29.05	0.00	18.75	0.00	24.99	0.00	22.22	0.00	75.00	0.00	26.80
481– 510	1.92	29.97	2.50	29.15	0.75	34.04	0.00	32.23	1.70	30.75	12.50	31.25	8.33	33.32	11.11	33.33	0.00	75.00	9.75	36.55
511– 540	1.37	31.34	1.66	30.81	4.54	38.58	0.00	32.23	2.01	32.76	6.25	37.50	0.00	33.32	11.11	44.44	0.00	75.00	4.87	41.42
541– 570	3.85	35.19	5.00	35.81	2.27	40.85	0.00	32.23	3.56	36.32	0.00	37.50	8.33	41.65	0.00	44.44	0.00	75 00	2.43	43.85
571– 600	3.03	38.22	2.50	38.31	1.51	42.36	0.00	32.23	2.47	38.79	0.00	37.50	0.00	41.65	0.00	44.44	0.00	75.00	0.00	43.85
601– 630	2.20	40.42	3.33	41.64	4.54	46.90	6.45	38.68	3.09	41.88	12.50	50.00	8.33	49.98	0.00	44.44	0.00	75.00	7.31	51.16
631– 660	3.85	44.27	5.00	46.64	3.78	50.68	16.12	54.80	4.64	46.52	0.00	50.00	0.00	49.98	0.00	44.44	0.00	75.00	0.00	51.16
661– 690	3.58	47.85	5.83	52.57	3.78	54.46	3.22	58.02	4.02	50.54	12.50	62.50	8.33	58.31	11.11	55.55	0.00	75.00	9.75	60.91
691– 720	4.40	52.25	3.33	55.80	2.27	56.73	0.00	58.02	3.56	54.10	0.00	62.50	0.00	58.31	0.00	55.55	0.00	75.00	0.00	60.91
721– 750	2.75	55.00	5.83	61.63	3.03	59.76	3.22	61.24	3.40	57.50	12.50	75.00	0.00	58.31	11.11	66.66	0.00	75.00	7.31	68.22
751– 780	3.30	58.30	0.83	62.46	0.00	59.76	3.22	64.46	2.16	59.66	6.25	81.25	0.00	58.31	11.11	77.77	0.00	75.00	4.87	73.09
781– 810	5.50	63.80	2.50	64.96	2.27	62.03	3.22	69.68	4.17	63.83	12.50	93.75	8.33	66.64	11.11	88.88	0.00	75.00	9.75	82.84
811– 840	5.78	69.58	4.16	69.12	6.06	68.09	6.45	74.13	5.57	69.40	0.00	93.75	8.33	74.97	0.00	88.88	0.00	75.00	2.43	85.27
841– 870	5.50	75.08	5.00	74.12	5.30	73.39	3.22	77.35	5.26	74.66	0.00	93.75	0.00	74.97	0.00	88.88	0.00	75.00	0.00	85.27
871– 900	5.50	80.58	1.66	75.78	6.06	79.45	3.22	80.57	4.79	79.45	0.00	93.75	8.33	83.30	0.00	88.88	25.00	100.00	4.87	90.14
901– 930	5.50	86.08	8.33	84.11	4.54	83.99	3.22	83.79	5.72	85.17	6.25	100.00	16.66	100.00	11.11	100.00			9.75	100.00
931– 960	6.61	92.69	4.16	88.27	7.57	91.56	6.45	90.24	6.34	91.51										
961– 990	3.58	96.27	6.66	94.93	4.54	96.10	3.22	93.46	4.33	95.84										
991–1020	2.75	99.02	1.66	96.59	3.03	99.13	3.22	96.68	2.63	98.47										
1021–1050	0.27	99.29	1.66	98.25	0.75	100.00	3.22	100.00	0.77	99.24										
1051–1080	0.55	100.00	0.83	99.08					0.46	99.70										
1081–1110			0.83	100.00					0.15	100.00										

TABLE 13

Bd F_2 *generation.*

AGE GROUP	DEATHS		NO. ALIVE AT START OF PERIOD	NO. WHICH LATER FORMED TUMORS	PERCENTAGE WHICH FORMED TUMORS
	TUMOR	NON-TUMOR			
211– 240	0	2	687	41	5.96
241– 270	0	1	685	41	5.98
271– 300	0	5	684	41	5.99
301– 330	0	13	679	41	6.03
331– 360	3	28	666	41	6.15
361– 390	3	31	635	38	5.98
391– 420	3	35	601	35	5.82
421– 450	2	45	563	32	5.68
451– 480	0	28	516	30	5.81
481– 510	4	11	488	30	6.14
511– 540	2	13	473	26	5.49
541– 570	1	23	458	24	5.24
571– 600	0	16	434	23	5.29
601– 630	3	20	418	23	5.50
631– 660	0	30	395	20	5.06
661– 690	4	26	365	20	5.47
691– 720	0	23	335	16	4.77
721– 750	3	22	312	16	5.12
751– 780	2	14	287	13	4.52
781– 810	4	27	271	11	4.05
811– 840	1	36	240	7	2.91
841– 870	0	34	203	6	2.95
871– 900	2	31	169	6	3.55
901– 930	4	37	136	4	2.94
931– 960	0	41	95		0.00
961– 990	0	28	54		0.00
991–1020	0	17	26		0.00
1021–1050	0	5	9		0.00
1051–1080	0	3	4		0.00
1081–1110	0	1	1		0.00

The numbers observed and the difference between the two crosses are sufficient to establish the point at issue without recourse to mathematical calculation of the probabilities. At the same time the resemblance between the two *dB* generations on one hand and the two *Bd* generations on the other is sufficiently close to make it obvious that within the particular type of reciprocal cross made the F_1 and F_2 generations are essentially similar. There is no sign of segregation between F_1 and F_2 as would be expected if Mendelian inheritance was involved to any great degree.

In the discussion, the influence of age as a possible means of explaining the differences will have to be taken up. It will be desirable to refer, for each of the F_2 generations to the percentages of deaths in each age-group and the cumulative percentages of tumor incidence and of death as was

TABLE 14

dB F_2 generation.

AGE IN DAYS	B AND b ANIMALS CONTRASTED												D AND d ANIMALS CONTRASTED											
	B, NON-TUMOR			b, NON-TUMOR			B, TUMOR			b, TUMOR			D, NON-TUMOR			d, NON-TUMOR			D, TUMOR			d, TUMOR		
		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE
211– 240	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	1	1.51	1.51	0	0.00	0.00	0	0.00	0.00	1	0.54	0.54	0	0.00	0.00
241– 270	0	0.00	0.00	1	0.92	0.92	1	0.58	0.58	0	0.00	1.51	1	0.30	0.30	0	0.00	0.00	1	0.54	1.08	0	0.00	0.00
271– 300	4	1.25	1.25	0	0.00	0.92	1	0.58	1.16	0	0.00	1.51	3	0.91	1.21	1	1.01	1.01	1	0.54	1.62	0	0.00	0.00
301– 330	45	14.06	15.31	7	6.48	7.40	5	2.94	4.10	5	7.57	9.08	39	11.85	13.06	13	13.13	14.14	8	4.39	6.01	2	3.70	3.70
331– 360	35	10.93	26.24	14	12.96	20.36	10	5.88	9.98	3	4.54	13.62	40	12.15	25.21	9	9.09	23.23	9	4.94	10.95	4	7.40	11.10
361– 390	35	10.93	37.17	18	16.66	37.02	11	6.47	16.45	4	6.06	19.68	49	14.89	40.10	4	4.04	27.27	15	8.24	19.19	0	0.00	11.10
391– 420	29	9.06	46.23	5	4.62	41.64	10	5.88	22.33	7	10.60	30.28	30	9.11	49.21	4	4.04	31.31	15	8.24	27.43	2	3.70	14.80
421– 450	12	3.75	49.98	4	3.70	45.34	13	7.64	29.97	3	4.54	34.82	12	3.64	52.85	4	4.04	35.35	15	8.24	35.67	1	1.85	16.65
451– 480	7	2.18	52.16	3	2.77	48.11	7	4.11	34.08	2	3.03	37.85	8	2.43	55.28	2	2.02	37.37	5	2.74	38.41	4	7.40	24.05
481– 510	4	1.25	53.41	2	1.85	49.96	11	6.47	40.55	4	6.06	43.91	4	1.21	56.49	2	2.02	39.39	10	5.49	43.90	5	9.25	33.30
511– 540	5	1.56	54.97	2	1.85	51.81	13	7.64	48.19	4	6.06	49.97	5	1.51	58.00	2	2.02	41.41	15	8.24	52.14	2	3.70	37.00
541– 570	8	2.50	57.47	0	0.00	51.81	9	5.29	53.48	4	6.06	56.03	5	1.51	59.51	3	3.03	44.44	9	4.94	57.08	4	7.40	44.40
571– 600	12	3.75	61.22	3	2.77	54.58	12	7.05	60.53	1	1.51	57.54	12	3.64	63.15	3	3.03	47.47	11	6.04	63.12	2	3.70	48.10
601– 630	16	5.00	66.22	4	3.70	58.28	10	5.88	66.41	4	6.06	63.60	15	4.55	67.70	5	5.05	52.52	10	5.49	68.61	4	7.40	55.50
631– 660	11	3.43	69.65	5	4.62	62.90	8	4.70	71.11	3	4.54	68.14	14	4.25	71.95	2	2.02	54.54	7	3.84	72.45	4	7.40	62.90
661– 690	8	2.50	72.15	2	1.85	64.75	8	4.70	75.81	7	10.60	78.24	6	1.82	73.77	4	4.04	58.58	6	3.29	75.74	9	16.66	79.56
691– 720	8	2.50	74.65	3	2.77	67.52	6	3.52	79.33	2	3.03	81.77	9	2.73	76.50	2	2.02	60.60	7	3.84	79.58	1	1.85	81.41
721– 750	10	3.12	77.77	5	4.62	72.14	6	3.52	82.85	1	1.51	83.28	10	3.06	79.53	5	5.05	65.65	6	3.29	82.87	1	1.85	83.26
751– 780	13	4.06	81.83	6	5.55	77.69	8	4.70	87.55	2	3.03	86.31	15	4.55	84.08	4	4.04	69.69	9	4.94	87.81	1	1.85	85.11
781– 870	8	2.50	84.33	1	0.92	78.61	4	2.35	89.90	1	1.51	87.82	6	1.82	85.90	3	3.03	72.72	4	2.19	90.00	1	1.85	86.96
811– 840	9	2.81	87.14	3	2.77	81.38	4	2.35	92.25	2	3.03	90.85	9	2.73	88.63	3	3.03	75.75	3	1.64	91.64	3	5.55	92.51
841– 870	6	1.87	89.01	1	0.92	82.30	4	2.35	94.60	2	3.03	93.88	3	0.91	89.54	4	4.04	79.79	5	2.74	94.38	1	1.85	94.36
871– 900	8	2.50	91.51	5	4.62	86.92	2	1.17	95.77	2	3.03	96.91	10	3.03	92.57	3	3.03	82.82	3	1.64	96.02	1	1.85	96.21
901– 930	9	2.81	94.32	3	2.77	89.69	3	1.76	97.53	0	0.00	96.91	5	1.51	94.08	7	7.07	89.89	2	1.09	97.11	1	1.85	98.06
931– 960	10	3.12	97.44	4	3.70	93.39	1	0.58	98.11	2	3.03	100.00	8	2.43	96.51	6	6.06	95.95	2	1.09	98.20	1	1.85	100.00
961– 990	6	1.87	99.31	2	1.85	95.24	3	1.76	100.00				5	1.51	98.02	3	3.03	98.98	3	1.64	100.00			
991–1020	2	0.62	100.00	5	4.62	100.00							6	1.82	100.00	1	1.01	100.00						
	320			108			170			66			329			99			182			54		

TABLE 15

Bd F_2 generation.

TABLE 15

Bd F_2 generation.

AGE IN DAYS	B AND b ANIMALS CONTRASTED												D AND d ANIMALS CONTRASTED											
	B, NON-TUMOR			b, NON-TUMOR			B, TUMOR			b, TUMOR			D, NON-TUMOR			d, NON-TUMOR			D, TUMOR			d, TUMOR		
		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE		PERCENT DEAD IN EACH GROUP	PERCENT DEAD AT EACH AGE
211– 240	1	0.20	0.20	1	0.61	0.61	0	0.00	0.00	0	0.00	0.00	2	0.40	0.40	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
241– 270	1	0.20	0.40	0	0.00	0.61	0	0.00	0.00	0	0.00	0.00	1	0.20	0.60	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
271– 300	3	0.62	1.02	2	1.22	1.83	0	0.00	0.00	0	0.00	0.00	4	0.80	1.40	1	0.66	0.66	0	0.00	0.00	0	0.00	0.00
301– 330	12	2.48	3.50	1	0.61	2.44	0	0.00	0.00	0	0.00	0.00	10	2.02	3.42	3	1.98	2.64	0	0.00	0.00	0	0.00	0.00
331– 360	22	4.55	8.05	6	3.68	6.12	2	7.14	7.14	1	7.69	7.68	20	4.04	7.46	8	5.29	7.93	1	4.00	4.00	2	12.50	12.50
361– 390	21	4.34	12.39	10	6.13	12.25	2	7.14	14.28	1	7.69	15.38	21	4.24	11.70	10	6.62	14.55	3	12.00	16.00	0	0.00	12.50
391– 420	25	5.17	17.56	10	6.13	18.38	1	3.57	17.85	2	15.38	30.76	27	5.45	17.15	8	5.29	19.84	1	4.00	20.00	2	12.50	25.00
421– 450	35	7.24	24.80	10	6.13	24.51	1	3.57	21.42	1	7.69	38.45	35	7.07	24.22	10	6.62	26.46	0	0.00	20.00	2	12.50	37.50
451– 480	14	2.89	27.69	14	8.58	33.09	0	0.00	21.42	0	0.00	38.45	26	5.25	29.47	2	1.32	27.78	0	0.00	20.00	0	0.00	37.50
481– 510	10	2.07	29.76	1	0.61	33.70	3	10.71	32.13	1	7.69	46.14	8	1.61	31.08	3	1.98	29.76	3	12.00	32.00	1	6.25	43.75
511– 540	7	1.44	31.20	6	3.68	37.38	1	3.57	35.70	1	7.69	53.83	11	2.22	33.30	2	1.32	31.08	2	8.00	40.00	0	0.00	43.75
541– 570	20	4.14	35.34	3	1.84	39.22	1	3.57	39.27	0	0.00	53.83	17	3.43	36.73	6	3.97	35.05	0	0.00	40.00	1	6.25	50.00
571– 600	14	2.89	38.23	2	1.22	40.44	0	0.00	39.27	0	0.00	53.83	13	2.62	39.35	3	1.98	37.03	0	0.00	40.00	0	0.00	50.00
601– 630	12	2.48	40.71	8	4.90	45.34	3	10.71	49.98	0	0.00	53.83	14	2.82	42.17	6	3.97	41.00	2	8.00	48.00	1	6.25	56.25
631– 660	20	4.14	44.85	10	6.13	51.47	0	0.00	49.98	0	0.00	53.83	19	3.83	46.00	11	7.28	48.28	0	0.00	48.00	0	0.00	56.25
661– 690	20	4.14	48.99	6	3.68	55.15	3	10.71	60.69	1	7.69	61.52	18	3.63	49.63	8	5.29	53.57	3	12.00	60.00	1	6.25	62.50
691– 720	20	4.14	53.13	3	1.84	56.99	0	0.00	60.69	0	0.00	61.52	19	3.83	53.46	4	2.64	56.21	0	0.00	60.00	0	0.00	62.50
721– 750	17	3.51	56.64	5	3.06	60.05	2	7.14	67.83	1	7.69	69.21	14	2.82	56.28	8	5.29	61.50	3	12.00	72.00	0	0.00	62.50
751– 780	13	2.69	59.33	1	0.61	60.66	1	3.57	71.40	1	7.69	76.90	12	2.42	58.70	2	1.32	62.82	2	8.00	80.00	0	0.00	62.50
781– 810	23	4.76	64.09	4	2.45	63.11	3	10.71	82.11	1	7.69	84.59	23	4.64	63.34	4	2.64	65.46	3	12.00	92.00	1	6.25	68.75
811– 840	26	5.38	69.47	10	6.13	69.24	1	3.57	85.68	0	0.00	84.59	29	5.85	69.19	7	4.63	70.09	0	0.00	92.00	1	6.25	75.00
841– 870	26	5.38	74.85	8	4.90	74.14	0	0.00	85.68	0	0.00	84.59	27	5.45	74.64	7	4.63	74.72	0	0.00	92.00	0	0.00	75.00
871– 900	22	4.55	79.40	9	5.52	79.66	1	3.57	89.25	1	7.69	92.28	28	5.65	80.29	3	1.98	76.70	0	0.00	92.00	2	12.50	87.50
901– 930	30	6.21	85.61	7	4.29	83.95	3	10.71	100.00	1	7.69	100.00	26	5.25	85.54	11	7.28	83.98	2	8.00	100.00	2	12.50	100.00
931– 960	29	6.00	91.61	12	7.36	91.31							34	6.86	92.40	7	4.63	88.61						
961– 990	21	4.34	95.95	7	4.29	95.60							19	3.83	96.23	9	5.96	94.57						
991–1020	12	2.48	98.43	5	3.06	98.66							14	2.82	99.05	3	1.98	96.55						
1021–1050	3	0.62	99.05	2	1.22	100.00							2	0.40	99.45	3	1.98	98.53						
1051–1080	3	0.62	99.67										2	0.40	100.00	1	0.66	99.19						
1081–1110	1	0.20	100.00													1	0.66	100.00						
	483			163			28			13			495			151			25			16		

TABLE 16
dB F_2 generation.

AGE GROUP	BLACKS					BROWNS					DILUTE BLACKS					DILUTE BROWNS				
	DEATHS		NO. ALIVE AT START OF PERIOD	NO. WHICH LATER FORMED TUMORS	PERCENT WHICH FORMED TUMORS	DEATHS		NO. ALIVE AT START OF PERIOD	NO. WHICH LATER FORMED TUMORS	PERCENT WHICH FORMED TUMORS	DEATHS		NO. ALIVE AT START OF PERIOD	NO. WHICH LATER FORMED TUMORS	PERCENT WHICH FORMED TUMORS	DEATHS		NO. ALIVE AT START OF PERIOD	NO. WHICH LATER FORMED TUMORS	PERCENT WHICH FORMED TUMORS
	TUMOR	NON-TUMOR				TUMOR	NON-TUMOR				TUMOR	NON-TUMOR				TUMOR	NON-TUMOR			
211– 240	0	0	374	130	34.75	1	0	137	52	37.95	0	0	116	40	34.48	0	0	37	14	37.83
241– 270	1	0	374	130	34.75	0	1	136	51	37.50	0	0	116	40	34.48	0	0	37	14	37.83
271– 300	1	3	373	129	34.58	0	0	135	51	37.77	0	1	116	40	34.48	0	0	37	14	37.83
301– 330	4	33	369	128	34.68	4	6	135	51	37.77	1	12	115	40	34.78	1	1	37	14	37.83
331– 360	7	29	332	124	37.34	2	11	125	47	37.60	3	6	102	39	38.23	1	3	35	13	37.14
361– 390	11	32	296	117	39.52	4	17	112	45	40.17	0	3	93	36	38.70	0	1	31	12	38.40
391– 420	9	25	253	106	41.89	6	5	91	41	45.05	1	4	90	36	40.00	1	0	30	12	40.00
421– 450	12	10	219	97	44.29	3	2	80	35	43.75	1	2	85	35	41.17	0	2	29	11	37.93
451– 480	3	5	197	85	43.14	2	3	75	32	42.66	4	2	82	34	41.46	0	0	27	11	40.74
481– 510	7	3	189	82	43.38	3	1	70	30	42.85	4	1	76	30	39.47	1	1	27	11	40.74
511– 540	11	4	179	75	41.89	4	1	66	27	40.90	2	1	71	26	36.61	0	1	25	10	40.00
541– 570	6	5	164	64	39.02	3	0	61	23	37.70	3	3	68	24	35.29	1	0	24	10	41.66
571– 600	10	10	153	58	37.90	1	2	58	20	34.48	2	2	62	21	33.87	0	1	23	9	39.13
601– 630	7	13	133	48	36.09	3	2	55	19	34.54	3	3	58	19	32.75	1	2	22	9	40.90
631– 660	5	9	113	41	36.28	2	5	50	16	32.00	3	2	52	16	30.76	1	0	19	8	42.10
661– 690	3	5	99	36	36.36	3	1	43	14	32.55	5	3	47	13	27.65	4	1	18	7	38.88
691– 720	6	6	91	33	36.26	1	3	39	11	28.20	0	2	39	8	20.51	1	0	13	3	23.07
721– 750	6	7	79	27	34.17	0	3	35	10	28.57	0	3	37	8	21.62	1	2	12	2	16.66
751– 780	7	9	66	21	31.81	2	6	32	10	31.25	1	4	34	8	23.52	0	0	9	1	11.11
781– 810	3	5	50	14	28.00	1	1	24	8	33.33	1	3	29	7	24.13	0	0	9	1	11.11
811– 840	1	7	42	11	26.19	2	2	22	7	31.81	3	2	25	6	24.00	0	1	9	1	11.11
841– 870	3	2	34	10	29.41	2	1	18	5	27.77	1	4	20	3	15.00	0	0	8	1	12.50
871– 900	1	7	29	7	24.13	2	3	15	3	20.00	1	1	15	2	13.00	0	2	8	1	12.50
901– 930	2	4	21	6	28.57	0	1	10	1	10.00	1	5	13	1	7.69	0	2	6	1	16.66
931– 960	1	5	15	4	26.66	1	3	9	1	11.11	0	5	7	0	0.00	1	1	4	1	25.00
961– 990	3	4	9	3	33.33	0	1	5	0	0.00	0	2	2	0	0.00	0	1	2	0	0.00
991–1020	0	2	2	0	0.00	0	4	4	0	0.00	0	0	0	0	0.00	0	1	1	0	0.00
1021–1050																				

TABLE 17

TABLE 17
Bd F_2 generation.

AGE GROUP	BLACKS					BROWNS					DILUTE BLACKS					DILUTE BROWNS				
	DEATHS		NO. ALIVE AT START OF PERIOD	NO. WHICH LATER FORMED TUMORS	PERCENT WHICH FORMED TUMORS	DEATHS		NO. ALIVE AT START OF PERIOD	NO. WHICH LATER FORMED TUMORS	PERCENT WHICH FORMED TUMORS	DEATHS		NO. ALIVE AT START OF PERIOD	NO. WHICH LATER FORMED TUMORS	PERCENT WHICH FORMED TUMORS	DEATHS		NO. ALIVE AT START OF PERIOD	NO. WHICH LATFR FORMED TUMORS	PERCENT WHICH FORMED TUMORS
	TUMOR	NON-TUMOR				TUMOR	NON-TUMOR				TUMOR	NON-TUMOR				TUMOR	NON-TUMOR			
211– 240	0	1	379	16	4.22	0	1	141	9	6.38	0	0	132	12	9.09	0	0	35	4	11.42
241– 270	0	1	378	16	4.23	0	0	140	9	6.42	0	0	132	12	9.09	0	0	35	4	11.42
271– 300	0	2	377	16	4.24	0	2	140	9	6.42	0	1	132	12	9.09	0	0	35	4	11.42
301– 330	0	9	375	16	4.26	0	1	138	9	6.52	0	3	131	12	9.16	0	0	35	4	11.42
331– 360	1	16	366	16	4.37	0	4	137	9	6.56	1	6	128	12	9.37	1	2	35	4	11.42
361– 390	2	14	349	15	4.29	1	7	133	9	6.76	0	7	121	11	9.09	0	3	32	3	9.37
391– 420	0	18	333	13	3.90	1	9	125	8	6.40	1	7	114	11	9.64	1	1	29	3	10.34
421– 450	0	28	315	13	4.12	0	7	115	7	6.08	1	7	106	10	9.43	1	3	27	2	7.40
451– 480	0	13	287	13	4.52	0	13	108	7	6.48	0	1	98	9	9.18	0	1	23	1	4.34
481– 510	2	7	274	13	4.74	1	1	95	7	7.36	1	3	97	9	9.27	0	0	22	1	4.54
511– 540	1	5	265	11	4.15	1	6	93	6	6.45	0	2	93	8	8.60	0	0	22	1	4.54
541– 570	0	14	259	10	3.86	0	3	86	5	5.81	1	6	91	8	8.79	0	0	22	1	4.54
571– 600	0	11	245	10	4.08	0	2	83	5	6.02	0	3	84	7	8.33	0	0	22	1	4.54
601– 630	2	8	234	10	4.27	0	6	81	5	6.17	1	4	81	7	8.64	0	2	22	1	4.54
631– 660	0	14	224	8	3.57	0	5	75	5	6.66	0	6	76	6	7.89	0	5	20	1	5.00
661– 690	2	13	210	8	3.80	1	5	70	5	7.14	1	7	70	6	8.57	0	1	15	1	6.66
691– 720	0	16	195	6	3.07	0	3	64	4	6.25	0	4	62	5	8.06	0	0	14	1	7.14
721– 750	2	10	179	6	3.35	1	4	61	4	6.55	0	7	58	5	8.62	0	1	14	1	7.14
751– 780	1	12	167	4	2.39	1	0	56	3	5.35	0	1	51	5	9.80	0	1	13	1	7.69
781– 810	2	20	154	3	1.94	1	3	55	2	3.63	1	3	50	5	10.00	0	1	12	1	8.33
811– 840	0	21	132	1	0.75	0	8	51	1	1.96	1	5	46	4	8.69	0	2	11	1	9.09
841– 870	0	20	111	1	0.90	0	7	43	1	2.32	0	6	40	3	7.50	0	1	9	1	11.11
871– 900	0	20	91	1	1.09	0	8	36	1	2.77	1	2	34	3	8.82	1	1	8	1	12.50
901– 930	1	20	71	1	1.40	1	6	28	1	3.57	2	10	31	2	6.45	0	1	6	1	16.66
931– 960	0	24	50	0	0.00	0	10	21	0	0.00	0	5	19	0	0.00	0	2	5	0	0.00
961– 990	0	13	26	0	0.00	0	6	11	0	0.00	0	8	14	0	0.00	0	1	3	0	0.00
991–1020	0	10	13	0	0.00	0	4	5	0	0.00	0	2	6	0	0.00	0	1	2	0	0.00
1021–1050	0	1	3	0	0.00	0	1	1	0	0.00	0	2	4	0	0.00	0	1	1	0	0.00
1051–1080	0	2	2	0	0.00	0	0	0	0		0	1	2	0	0.00					
1081–1110											0	1	1	0	0.00					

done in tables 1, 3, 4, and 6 for dilute browns, blacks and the two F_1's respectively.

These data are, as previously indicated, given in tables 8 and 9 for the *dB* F_2 generation and in tables 11 and 12 for the *Bd* F_2 generation.

In each case, color classes and tumor and non-tumor mice are kept distinct. Actual incidence of mice in each 30-day age-group as well as the cumulative percentage at each successive age are given.

The F_2 generations also are tabulated separately with the two pairs of factors *B-b* and *D-d* used as the basis of comparison. Tumor and non-tumor animals are kept distinct so that they may later be compared. These data for the *dB* F_2 generation are shown in table 14 and for the *Bd* F_2 generation in table 15.

In order that curves may later be constructed, the data for the tumor rate in the two F_2 generations, divided according to coat color, is given in table 16 for the *dB* F_2 generation and in table 17 for the *Bd* F_2 generation.

The primary data tabulated in these seventeen tables may properly serve as a basis for discussion of the results.

DISCUSSION

It should be remembered at all times that the incidence of mammary tumors has little or none of the definiteness that characterizes the appearance of such distinct genetic characters as the basic coat colors or the more simple morphological abnormalities.

By considering only virgin females in the hybrid generation the possible complicating factor of variation in development of the mammary structure has been reduced to a minimum consistent with avoiding any surgical mutilation of the animals.

The incidence of mammary tumors in any generation has, for practical purposes, a minimum age limit below which no tumors have been observed on the living mouse. The detection of a tumor by palpation is sufficiently delicate to compare favorably with those methods used ordinarily in the observation of any of the usual morphological abnormalities. It is as accurate a means of determining the existence of a tumor without harming the individual, as has been devised. It has been employed throughout these experiments.

In comparing the relative incidence of tumors in any two populations the age distribution of tumors observed becomes an important factor.

It is obvious that the age at which the earliest recorded tumor appears may be taken as a threshold below which animals cannot be classified with any degree of accuracy. This fact is commonly recognized.

As an animal grows older its chance of developing a tumor increases. It will be helpful, if for each comparable population of animals we can

arrive at some measure of this increase in probability of tumor formation as the age of the individual increases.

An animal which fails to develop a tumor represents an unfulfilled opportunity. Its failure to develop a tumor may depend on the fact that it is constitutionally of a type which will not do so. On the other hand its failure to develop a tumor may be due to the fact that it has died at an age at which few of the animals of its generation destined to produce tumors have done so.

The distribution of mammary tumor mice in any given generation is our best indication of the relation between age and mammary tumor production. It can be used as a standard from which the probability that any mouse of that generation has of producing a mammary tumor, may be calculated.

It is evident that a mouse which lives to an advanced age-group has had all the chance of developing a mammary tumor which has been expressed up to the particular age-group at which it dies.

Proceeding from this line of reasoning the following conclusions may be reached. The unfulfilled chance which a non-tumor mouse has had of developing mammary tumor may be expressed by the percentage of mammary tumors within the generation to which it belongs, that developed in the various age-groups through which the non-tumor mouse has passed.

This process by the method described under the section dealing with the dilute brown parent stock the unfulfilled probability of developing tumors exhibited by any population can be fairly compared with other populations and a measure of the influence of age obtained. The population with the highest fractional value has had the best chance—as far as age is concerned—of developing tumors. In establishing a standard with which the age percentage distributions of any particular population is to be compared, it seems proper, as before mentioned, to select as the earliest age-group to be included in the data for any generation or color class, that one in which the youngest animal with mammary tumor is recorded. If then the reciprocal generations have an unequal distribution of tumor animals, one for example including a tumor at an earlier age-group than the other, something must be done to correct for that discrepancy before a direct comparison between the two reciprocal generations is made. It is evident that to do this by combining the actual numbers of the two tumor populations would result in giving undue importance to the cross having the larger number of animals. To avoid this, percentages in the various age classes must be used both for tumor and non-tumor mice. The most satisfactory way seems to be to combine the tumor distributions of the two groups by adding together the percentages of tumor mice occurring in successive age-groups of both, dividing by two and calculating the cumula-

tive incidence of tumors on the basis of the results thus obtained. This is done for all the populations to be compared, in table 18. The particular population involved is indicated in each case in that table.

The first populations to be compared are those showing each of the two alleles of each of the color pairs involved, *B-b* and *D-d*.

Reference to the section on experimental results will show that in each F_2 generation animals phenotypically brown (*b*) developed a slightly higher percentage of tumors than those which were black (*B*). This difference amounted to 3.24 percent in the *dB* F_2 where the figures were 37.93 percent for browns and 34.69 percent for blacks. In the *Bd* F_2 generation the percentages were 5.47 for blacks and 7.38 for browns, a difference of 1.91 percent. In themselves, these differences are not large but they might possibly indicate a trend and because of that fact must be evaluated in the light of the age of the populations in which they occur.

Calculations of the age distribution of the black (*B*) animals of *dB* F_2 in relation to the age at which mammary tumors occurred in that color and generation gives a value of 3633.37 or expressed as a decimal .3633 where unity is the survival of all non-tumor animals beyond the age at which the last tumor appeared. A similar calculation for the brown (*b*) animals of *dB* F_2 gives a value of .4458. The brown animals thus had a better chance to develop tumors than did the blacks of that generation. This may partially or wholly explain the difference which was observed.

When the *Bd* F_2 generation is considered, a definitely higher value is obtained. Black (*B*) animals give .5826 and browns (*b*) .5928. Again the browns have survived relatively longer than the blacks and thus have had a better chance of forming tumors.

It seems therefore wise to conclude that the slight excess of tumors in browns of both F_2 generations does not denote any genetic linkage between (*b*) and any gene or genes for the formation of mammary tumors.

A similar study of the F_2 populations of *D* intense and *d* dilute animals is also of interest.

In this case the *dB* F_2 intense mice give a value of .3637 and the dilute mice .4761. The percentage of tumor mice in these two color types was very nearly equal being 35.61 percent for intense animals and 35.29 percent for dilute. Interpreted in terms of age the dilute animals had a distinctly better chance to develop tumors than did the intense.

In the *Bd* F_2 generation, however, the mammary tumor incidence in intense animals was 4.80 percent and in dilutes 9.58 percent. This difference is further accentuated when age is considered. The value for intense animals is .5970 and for dilutes .5625. The most probable explanation for the difference in this case is that of the small number of animals on which the calculations are based. In the *Bd* F_2 generation only 41 mammary

tumors were recorded. Of these 25 were in intense and 16 in dilute mice. In spite of the excess in incidence of mammary tumors in dilute animals of this generation, therefore, it is very doubtful whether genetic linkage involving one or more genes is involved. If it had been we should have expected the *dB* F_2 generation to provide supporting evidence which it clearly fails to do.

Another factor which may be related to coat color and which therefore requires further consideration is the rate at which the incidence of mammary tumors occurs. The method of tabulating data on the point is also described in the section on material under the discussion of the dilute brown (*dba*) high tumor stock.

For the F_2 generations these data, divided according to the various coat color classes, are given in tables 16 and 17 and are shown graphically in the form of charts in figure 1.

It should be remembered that each point shown represents the percent of animals dying of tumor at any date later than the beginning of the age class indicated by the point in question.

Reference to tables 16 and 17 will make clear the fact that the irregularities observed in the later age-groups are due to small numbers and the resulting large rise or fall of the curve, when even a single animal is added or subtracted.

Inspection alone, without the need of recourse to mathematical comparison of the curves, shows that insofar as each generation is concerned the different color classes resemble one another closely. There is no evidence that the rate of tumor incidence is correlated with the *B-b* or *D-d* pair of genes or that it is significantly divergent from the mean in any one color class.

It might be thought that the two dilute classes in the *dB* F_2 generation showed a tumor rate that fell off more rapidly than did the intense pigmented varieties. This tendency, if correlated physiologically or genetically with "dilution" should also manifest itself in the dilute classes of the *Bd* F_2. Such however is not the case. The general conclusion that the rate of tumor incidence is independent of the *B-b* and *D-d* pairs of genes, therefore, holds good.

Having considered and discarded the possibility that the recognizable genes for coat color involved in the cross disturb in any way the genetic relationships involved in the incidence of mammary tumors, we may safely proceed to compare the various generations without further reference to the coat color of the animals included in them.

COMPARISON OF THE F_1 GENERATIONS

In these two generations all animals are black in color. Theoretically they should be genetically similar and directly comparable. A composite

TABLE 18

Composite percentage distribution of mammary tumor incidence.

AGE GROUP IN DAYS	F_1 GENERATION		F_2 GENERATION TOTAL		B ANIMALS IN F_2		b ANIMALS IN F_2		D ANIMALS IN F_2		d ANIMALS IN F_2	
	PERCENT IN EACH AGE GROUP	PERCENT DEAD AT EACH AGE GROUP	PERCENT IN EACH AGE GROUP	PERCENT DEAD AT EACH AGE GROUP	PERCENT IN EACH AGE GROUP	PERCENT DEAD AT EACH AGE GROUP	PERCENT IN EACH AGE GROUP	PERCENT DEAD AT EACH AGE GROUP	PERCENT IN EACH AGE GROUP	PERCENT DEAD AT EACH AGE GROUP	PERCENT IN EACH AGE GROUP	PERCENT DEAD AT EACH AGE GROUP
151– 180	1.11	1.11										
181– 210	0.00	1.11										
211– 240	1.11	2.22	0.21	0.21	0.00	0.00	0.75	0.75	0.27	0.27	0.00	0.00
241– 270	0.00	2.22	0.21	0.42	0.29	0.29	0.00	0.75	0.27	0.54	0.00	0.00
271– 300	0.00	2.22	0.21	0.63	0.29	0.58	0.00	0.75	0.27	0.81	0.00	0.00
301– 330	1.11	3.33	2.11	2.74	1.47	2.05	3.78	4.53	2.19	3.00	1.85	1.85
331– 360	2.17	5.50	6.90	9.64	6.51	8.56	6.11	10.64	4.47	7.47	9.95	11.80
361– 390	3.28	8.78	6.83	16.47	6.80	15.36	6.87	17.51	10.12	17.69	0.00	11.80
391– 420	3.28	12.06	7.25	23.72	4.72	20.08	12.99	30.50	6.12	23.81	8.10	19.90
421– 450	5.55	17.61	5.82	29.54	5.60	25.68	6.11	36.61	4.12	27.93	7.17	27.07
451– 480	5.55	23.16	1.90	31.44	2.05	27.73	1.51	38.12	1.37	29.30	3.70	30.77
481– 510	4.44	27.60	8.05	39.49	8.59	36.32	6.87	44.99	8.74	38.04	7.75	38.52
511– 540	9.90	37.50	6.03	45.52	5.60	41.92	6.87	51.86	8.12	46.16	1.85	40.37
541– 570	0.00	37.50	3.96	49.48	4.43	46.35	3.03	54.89	2.47	48.63	6.82	47.19
571– 600	5.50	43.00	2.75	52.23	3.52	49.87	0.75	55.64	3.02	51.65	1.85	49.04
601– 630	4.39	47.39	6.62	58.15	8.29	58.16	3.03	58.67	6.74	58.39	6.82	55.86
631– 660	8.74	56.13	2.33	61.18	2.35	60.51	2.27	60.94	1.92	60.31	3.70	59.56
661– 690	2.22	58.35	8.05	69.23	7.70	68.21	9.14	70.08	7.64	67.95	11.45	71.01
691– 720	5.45	63.80	1.69	70.92	1.76	69.97	1.51	71.59	1.92	69.87	0.92	71.93
721– 750	4.39	68.19	4.63	75.55	5.33	75.29	4.60	76.19	7.64	77.51	0.92	72.85
751– 780	6.61	74.80	4.55	80.10	4.13	79.42	5.36	81.55	6.47	83.98	0.92	73.77
781– 810	3.28	78.08	5.93	86.03	6.53	85.95	4.60	86.15	7.09	91.07	4.05	77.82
811– 840	4.34	82.42	2.48	88.51	2.96	88.91	1.51	87.66	0.82	91.89	5.90	83.72
841– 870	2.17	84.59	1.27	89.78	1.17	90.08	1.51	89.17	1.37	93.26	0.92	84.64
871– 900	4.39	88.98	3.28	93.06	2.37	92.45	5.36	94.53	0.82	94.08	7.17	91.81
901– 930	2.17	91.15	5.51	98.57	6.23	98.68	3.84	98.37	4.54	98.62	7.17	98.98
931– 960	4.39	95.54	0.63	99.20	0.29	98.97	1.51	100.00	0.54	99.16	0.92	100.00
961– 990	2.17	97.71	0.63	100.00	0.88	100.00			0.82	100.00		
991–1020	0.00	97.71										
1021–1050	0.00	97.71										
1051–1080	0.00	97.71										
1081–1110	0.00	97.71										
1111–1140	2.17	100.00										

percentage distribution curve of tumor incidence has been constructed for them as in the case of the color groups of F_2 already considered. It is shown in the two first groups of table 18.

When the age of *dB* F_1 non-cancer animals is calculated on the basis of 20 of these data the value obtained is .3941. The value for the *Bd* F_1 generation is .4392. Whatever age difference there is, is in the direction of favoring mammary tumor formation in the *Bd* F_1. Since this generation produced only 6.06 percent of mammary tumors against 39.82 percent in the *dB* F_1 it is evident that the excess of tumors in that generation exists in spite of the age distribution of the animals comprising it rather than because of any favorable statistical influence of the age factor.

In order to determine whether the difference in tumor incidence in the two F_1 generations is a constant phenomenon occurring at all ages, the data given in tables 5 and 7 may be compared. This is done in figure 2 (solid lines). It is clear that the difference between the two F_1 generations is characteristic of each and every age group and that it cannot therefore be looked upon as a temporary or transitory phenomenon. The sudden rise in the last recorded age-group of the *Bd* F_1 generation has no significance since only a total of five mice, one of them tumorous, is involved.

It is also of interest to note the close approximation of the *dB* F_1 tumor incidence curve to that for virgin dilute brown (*dba*) stock females (figure 2 top line). It is clear that the F_1 hybrid animals of that cross (*dB* F_1) resemble the high tumor parent line closely.

In later discussion it will be shown that this relationship is probably to be distinguished definitely from that of Mendelian dominance. The large number of mammary tumors observed in the F_1 generation clearly prove that the tendency of the mammary gland to form tumors is in no way whatever to be considered a simple Mendelian recessive thus definitely confirming the conclusion reached by one of us (LITTLE 1928).

If the tendency to form mammary tumors were due to a simple Mendelian recessive gene there would be no such tumors formed in the F_1 generations derived from crossing together a "high cancer" and a "non-cancer" strain. Actually 68 such tumors have been obtained in the F_1 generation.

Further than this no F_2 generation in which a large number of animals has been raised should have more than 25 percent tumor. Actually the *dB* F_2 generation consisting of 664 animals shows 35.54 percent tumor.

We are, moreover, faced with a situation in which, having corrected for age, the reciprocal F_1's show markedly different incidence of mammary tumors. The chromosomal constitution of female mice of the two F_1's is theoretically identical. The factor of variation in tumor incidence due to pregnancy or lactation has been avoided by using only virgins.

There remains only extra-chromosomal influence correlated with the strain from which the female sex cells are derived to explain the observed differences.

The more detailed analysis of the completed crosses thus confirms entirely the preliminary note on this material to which earlier reference has been made.

Concerning the nature of the extra-chromosomal influence there are many possibilities. It seems wiser, however, to make no statement on this point until further investigations have been completed.

COMPARISON OF THE F_2 GENERATIONS

Since we have no evidence of any significant complications in the ordinary processes of Mendelian inheritance, insofar as the behavior of color genes in this material is concerned, and since age does not seem to modify to any statistically significant degree the relative incidence of mammary tumors in the various F_2 color classes, the F_2 generations may safely be analyzed by the same procedure as that applied to F_1.

The first step in this process is the construction of a composite percentage age distribution for the F_2 generation. This is shown in the third and fourth groups of table 18. On the basis of this distribution the *dB* F_2 generation gives a value of .3932 and the *Bd* F_2 generation .5883. The tumor incidence of these two generations is 35.54 percent and 5.96 percent respectively. Here, even more clearly than in the F_1 generation, the age factor favors tumor production in the low tumor generation *Bd* F_2.

COMPARISON OF THE F_1 AND F_2 GENERATIONS

By observation, the difference in the values of 39.82 percent mammary tumors in *dB* F_1 and 35.54 percent in *dB* F_2 is small enough to be considered due to chance. So also is the difference between the tumor incidence percentages of *Bd* F_1 6.06 and that of *Bd* F_2 5.96 respectively.

This fact is also clearly brought out in figure 1. The *dB* F_1 generation (solid line) may be directly compared with the *dB* F_2 (broken line). These two curves are very closely parallel to one another and indicate that there is a common explanation to be sought in both cases.

The same holds true for the *Bd* F_1 generation (solid line) and the *Bd* F_2 generation (lower broken line). There may be some evidence that the two F_2 generations are on the whole slightly less likely to form tumors than are their respective F_1's. They are however just as far removed from one another as are the two reciprocal F_1 types. In other words there is *no* evidence of any tendency for the difference to disappear.

As regards the early incidence of tumors there is some reason for stating that the *dB* F_2 begins to form tumors at a later age than the *dB* F_1. Whether this has real significance will be more easily determined when

data from backcross generations now being observed are complete. In the meanwhile, in view of the very close parallel between the age of tumor incidence in the *Bd* F_1 and *Bd* F_2 generations the difference observed between them and the *dB* crosses can be noted and kept in mind for further study without, at this time, attempting to make any estimate of its significance.

Whatever extra-chromosomal influence was operative in differentiating between the reciprocal F_1 generations, thus appears to have persisted and to have produced a similar result in reciprocal F_2 generations.

The incidence of mammary tumors in the *Bd* F_1 and *Bd* F_2 generations although low, is sufficient to keep open the possibility that a genetic influence, borne in the chromosomes, or dependent upon them, *may* play some role in the production of mammary tumors.

If it does, however, it is overshadowed by the directly transmitted extra-chromosomal influence which is approximately six times more important in the material studied.

It should also be noted that longevity, although not analyzed in detail, seems to show very much the same general behavior.

Since both the incidence of mammary tumors and longevity bear an obvious and close relationship to one another and to the aging of tissues within the body it would appear that an important principle may be involved.

This may be roughly stated somewhat as follows. In the fundamental physiological processes dependent upon the successful functioning of the mammalian organism as a whole, either through balance or unbalance of its component systems, direct transmission of extra-chromosomal and possibly cytoplasmic influences may be far more important than the chromosomal genes. The implications involved are of obvious importance to the relation of Mendelian heredity to practical eugenics, medicine and education.

SUMMARY AND CONCLUSIONS

Two inbred strains of mice, one of which (*dba*) gives an incidence of 50.84 percent spontaneous mammary tumors in virgin females, and the other of which, C57 black, has given no such tumors in either breeding or virgin females, have been used as parents for an outcross.

The cross has been made reciprocally and the following number of virgin females in each generation have been raised. These animals have been observed until the appearance of a mammary tumor or until their death from other causes, after they have reached "tumor age."

113 *dba* ♀ × C57 blk ♂ (*dB* F_1)
379 C57 blk ♀ × *dba* ♂ (*Bd* F_1)
664 (*dba* ♀ × C57 ♂)F_1 *inter se* (*dB* F_2)

687 (C57 ♀ × *dba* ♂)F_1 *inter se* (*Bd* F_2)
1843 Total all hybrid generations

After due allowance has been made for age of each population the following conclusions may be drawn.

(1) The *dB* F_1 animals show a significantly higher incidence of mammary tumors than do those of the *Bd* F_1 generation, 39.82 percent as against 6.06 percent.

(2) This difference persists in the reciprocal F_2 generation as follows: *dB* F_2 35.54 percent; *Bd* F_2 5.96 percent.

(3) Since the chromosomal constititution of the females of the reciprocal F_1 generations is theoretically the same, the observed difference in the incidence of mammary tumors must be due to extra-chromosomal influence.

(4) Since this difference persists in the two F_2 generations, the chromosomal make-up of which is theoretically the same, its chief basis is obviously transmitted in some manner by influence outside of the chromosomes.

(5) The occurrence of some mammary tumors in both F_1 and in both F_2 generations indicates that the possibility of some chromosomal influence in the genesis of mammary tumors is not excluded.

(6) The difference in tumor incidence in the reciprocal crosses of both F_1 and F_2 generations lasts throughout all age periods and is strikingly constant.

(7) There is no evidence of the linkage of such influence with any of the coat color genes (*B-b* or *D-d*) studied.

(8) The relative importance of what appears to be extra-chromosomal influence in the incidence of mammary tumors in the mice studied, is approximately six times that of the possible chromosomal influence.

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AN ANALYTICAL STUDY OF CROSSING OVER IN DROSOPHILA MELANOGASTER

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INTRODUCTION

In 1902, SUTTON pointed out the close analogy between Mendelian heredity and the cytological processes of chromosome reduction and fertilization. He clearly foresaw and predicted the discovery of linkage groups (1903). The discovery of linkage (BATESON, SAUNDERS AND PUNNETT 1906) and the formulation of the chiasmatype hypothesis (JANSSENS 1909) followed shortly thereafter. When linkage was discovered and analyzed in the sex chromosome of Drosophila in 1910, it was interpreted by MORGAN and his students in terms of the chiasmatype hypothesis (MORGAN 1911, MORGAN, STURTEVANT, MULLER and BRIDGES 1915, MULLER 1916).

With the accummulation of genetic data on Drosophila and other forms, additional concepts regarding the physical basis of linkage were developed. Chief among these were: (1) the linear order of the gene, (2) interchange between homologous chromosomes in blocks of genes, (3) interference, (4) chromosome maps, indicating the location of genes with respect to each other, and (5) the limitation of linkage groups.

With the discovery of double-strand (four strand) crossing over (BRIDGES 1916), the analysis of linkage data in attached X's (ANDERSON 1925, L. V. MORGAN 1925, STURTEVANT 1931, RHOADES 1931, EMERSON and BEADLE 1933), triploids (BRIDGES and ANDERSON 1925, REDFIELD 1930, 1932) and heteroploids generally, translocations, and with analysis of X-ray effects on mutation, etc., it has been recognized for some years (MORGAN, BRIDGES and STURTEVANT 1925, MULLER 1930, MULLER and PAINTER 1932, DOBZHANSKY 1930, 1931, 1932a, b, 1933, REDFIELD 1930, 1932, BRIDGES 1932a) that analyses, in terms of the leading concepts regarding the mechanism of crossing over, describe these phenomena only approximately. Recently, several workers (WEINSTEIN 1932, MATHER 1933, RHOADES 1933, EMERSON and RHOADES 1933, GRAUBARD 1934, SCHWEITZER 1934) have called attention to some theoretical changes that have become necessary in order to describe more adequately the recorded data on recombination among linked genes.

In this paper, some of the linkage data from *Drosophila melanogaster* will be analyzed with the object of elucidating certain further details of the cytological processes involved.

Many friends and co-workers have rendered assistance on numerous

occasions with the computations and interpretations, on which the following is based. For this the author wishes to make acknowledgement here. He is especially indebted to Mrs. L. V. MORGAN of the CALIFORNIA INSTITUTE OF TECHNOLOGY for permission to make use of data prior to publication, and to her and to Professor A. H. STURTEVANT of the CALIFORNIA INSTITUTE OF TECHNOLOGY, Doctor M. A. GRAUBARD and Professors D. E. LANCEFIELD and L. C. DUNN of COLUMBIA UNIVERSITY, for many helpful suggestions in connection with the following work.

THE POSTULATES OF CROSSING OVER

Ambiguity has arisen from the confused usage of the words recombination and crossing over, which are occasionally used as synonyms and at other times as distinct and different terms. BRIDGES has recently suggested (1932a) that the term crossing over should refer to the cytological mechanism, whereas recombination has reference to the empirical results obtained from an F_2 or a backcross. The procedure that will be followed in this paper will be to refer to the cytological events as crossing over and to the genetic results as recombination of linked genes.

Several of the concepts referred to have been sufficiently verified and require little additional discussion.

1. The work of STERN 1931, CREIGHTON and MCCLINTOCK 1931, and RHOADES 1932, demonstrates that genetic recombination is accompanied by cytological crossing over.

2. Extensive analyses of recombination data have shown that the exchange of genes between homologous chromosomes does indeed occur in blocks (STURTEVANT 1913, 1915, MORGAN, BRIDGES and STURTEVANT 1925).

3. The recent findings with induced translocations and deficiencies verify the linear order of the genes, as postulated (MULLER and PAINTER 1929, DOBZHANSKY 1929).

4. The correspondence between the number of chromosomes and the number of linkage groups has also been established in the forms most thoroughly investigated (see especially MORGAN, BRIDGES and STURTEVANT 1925, STERN 1927, R. A. EMERSON 1932, etc.).

Our understanding of the chromosome maps and our concepts of coincidence and interference need to be modified, however, in order for them to continue to be useful in elucidating the mechanism of crossing over from data on the recombination of linked genes.

COINCIDENCE AND INTERFERENCE

Recombinations involving two regions in a chromosome simultaneously generally occur less frequently than are to be expected from the known

incidence of recombination in each region separately. The comparison between the number obtained and those expected has been expressed as a ratio obtained/expected, which is known as the coincidence ratio or index. The cytological events which apparently underly the variable coincidence index may be stated as follows: simultaneous double (and multiple) recombinations occur less frequently than are to be expected on a chance basis in nearby regions and are progressively less frequent as the distance between the breaks diminishes. This relation has usually been expressed as the interference of one cross over on the occurrence of another nearby.

Certain difficulties have appeared in the use of the coincidence index as a measure of interference:

(1) Coincidence values differ in certain critical cases depending on whether the calculations are made as complete coincidence (MULLER 1916) or as partial coincidence (WEINSTEIN 1918). This has recently been indicated by WEINSTEIN (1932) and by the present writer (1934).

(2) The indices obtained when computed for two regions with fairly long, intermediate genetic distances will differ when calculated from different sets of data depending on the number of *undetected* double crossovers that occurred within the limits of the regions being considered in the particular experiment from which the computations are made (SCHWEITZER, loc. cit.).

(3) No simple theoretical interpretation of coincidence can be given because of the way in which the data are grouped. The usual procedure is to lump together in the non-crossover class all flies which do not give recombination in at least one of the two regions under consideration. For example, in a cross involving genes *a b c d e f g* (denoting the regions by numbers, that is, $a-b=1$, $b-c=2$, etc.) to calculate coincidence for *a-b* and *d-e* (that is, 1, 4) all the 2, 3, 5, and 6 flies are treated as non-crossovers in the data. Similarly, 1, 2 flies are treated as 1, etc. and 1, 4, 6 flies are grouped with the 1, 4 class. But it is known that recombination in regions 2, 3, 5 or 6 do themselves restrict the incidence of simultaneous exchanges in region 1 and region 4. Consequently, coincidence, as measured, bears no simple relation to the cytological forces of interference of which it (coincidence) is a result.

In my previous paper, a method for the direct expression of interference relations between any two regions is given. When computed by this method, only those classes having direct reference to the events in the two particular regions being studied are included. In the example previously cited, classes 2, 3, 5 and 6 and all multiples except 1, 4 are excluded entirely from consideration. Only four classes are used, the non-crossover class, the class giving recombination for region 1 only, the class giving recombination for region 4 only, and the double crossover class 1, 4. The ratio calculated is called *fraction realized*.

RECOMBINATION VALUES AND CHROMOSOME MAPS

The construction of genetic chromosome maps

The construction of chromosome maps with numerical values has been in units of recombination percent. The distance between two genes on a chromosome has been defined as the sum of the recombination percents of the loci included between them, taken two at a time. For example, for genes *a b c d e f g*, the distance $a-g$ is equal to the sum of $a+b$, and $b+c$, etc. . . . and $f+g$ (BRIDGES and MORGAN 1923).

It was recognized and stated when even the earliest genetic maps of the X chromosome of Drosophila were made by STURTEVANT in 1913, that these maps did not necessarily accurately represent the spatial relations of the genes (STURTEVANT 1913, MORGAN, STURTEVANT, MULLER and BRIDGES 1915), and that any influence that would tend to modify the recombination values would thereby change the positions of the relevant loci on the maps, and by the same amount. It has been considered likely that the different degrees of crowding of genes that appear in different regions of the maps represented differences in crossover values of different parts of the chromosome, rather than differences in mutation rate, etc. (MORGAN, BRIDGES and STURTEVANT 1925, page 91 on, DOBZHANSKY 1929).

Chromosome maps and the cytological alignment of genes

In the tetrad, three perhaps independent sets of conditions are involved, of which recombination of linked genes is observed as the end result. These are synapsis, exchange and disjunction. Changes in any one will affect recombination values. When crossing over is discussed, the reference is apparently usually intended to refer to the mechanism of exchange, and all differences in percentage of recombination are referred to the proximity of the spindle fibre or to the distances between the loci, these two factors having been primarily considered as responsible for differences in recombination. In addition, in a form like Drosophila, the normal recovery of only one chromatid of a given tetrad must be taken into account. While it is true that every case of recombination of linked genes represents an antecedent event of exchange, every single or multiple exchange in a tetrad does not necessarily yield an offspring with an equal number of recombinations. This is so because only two of the four strands are involved at any level and only one strand of any tetrad is normally recovered, and furthermore, there may be a correlation between exchange and disjunction (WEINSTEIN 1932, R. EMERSON and RHOADES 1933, S. EMERSON and BEADLE 1933, L. V. MORGAN 1933, MORGAN, BRIDGES and SCHULTZ 1933). It may be accepted, therefore, that there is not a direct and simple relation between the genetic maps and the spatial arrangement of the genes.

The results of several lines of investigation, and especially the study of cytological configurations derived from translocations induced by X-rays (PAINTER and MULLER 1930, 1932, MULLER and ALTENBERG 1930, DOBZHANSKY 1930a, 1931) have supported these doubts regarding the accuracy of the distances on the genetic chromosome maps.

Recently, several new types of maps of the chromosomes of Drosophila have been published, differing in certain respects from the old maps. (1) REDFIELD has published a genetic map of the II and III chromosomes, based on data from triploids. These show less crowding of the genes than the ordinary genetic maps, but being similarly derived, these maps have the same general weaknesses as the old maps. In addition, the comparison between diploid and triploid maps is inadequate, because in the triploid offspring only one strand from a hexad is normally recovered, while in the offspring of diploids one quarter of the tetrad strands are obtained. In the triploid also, the possible frequent changes of partner in synapsis must be taken into account when comparisons are made with the diploid. (2) DOBZHANSKY (1929, 1930, 1931, 1932) and PAINTER and MULLER (1929a, b, 1932) have published approximate maps based on studies of metaphase plates involving fragments and translocations obtained in X-ray experiments. These maps probably represent a closer approximation to the precrossing over condition, but there are certain defects in their construction which render them unsuitable for quantitative analyses: (a) they are based on metaphase configurations, and the degree of contraction for various parts of the chromosome is not known, but can scarcely be assumed to be uniform; (b) they are based on studies of chromosomes of very small total length, and measurements cannot be made with high accuracy; (c) they are based on few loci and are therefore not susceptible to quantitative treatment. (3) MULLER has constructed a map of the X chromosome, in which the distances between loci are proportional to the frequencies with which mutations have been found in the regions between them ("mutation frequency" map) see MULLER and PAINTER 1932, pp. 321–8.

The basis for the construction of this mutation frequency map will be considered in some detail because of its great utility in reconstructing some of the events whose results we commonly observe as recombination of linked genes.

It is commonly assumed among geneticists that the genes hitherto found represent a random sample of all the genes in the chromosomes, and that, with the exception of regions demonstrated to be largely inert, genes are probably uniformly distributed throughout the lengths of the chromosomes. It is also generally assumed that the genes already reported are representative samples of the total gene population. To these may be added a third postulate, namely, that differences in mutation rate of various loci are not correlated with their positions in the chromosomes.

With these assumptions in mind, if all the genes which have been reported are arranged in their known order, the relative distance between any two genes may be expressed as the fraction of all the genes in that chromosome which lie between them.

The construction and use of the leptotene maps

The maps used in this paper differ in certain respects from the one drawn by MULLER (MULLER and PAINTER, loc. cit. 1932). For calculating the "mutation frequency" map, MULLER used the total number of mutations, and for the chromosome he used, namely the X, 570 mutations were available for inclusion in this map, for the most part lethals from X-ray experiments. In his map, however, rapidly mutating loci appear more frequently than do those with a lower rate of mutation, and multiple alleles are apparently counted each as a separate mutation. It seemed desirable to correct for these factors, and also to limit the cases selected to known point mutations. With these factors in mind, a census of all reported mutations on the X, II and III of *Drosophila melanogaster* was prepared. All cases which were suspected or known to be deficiencies or other than point mutations were eliminated. Multiple alleles and rapidly mutating genes were each counted only once. This method gives the closest approximation to a random sample at present available. As used in this paper, there were available 97 loci for the X chromosome, 88 for the II chromosome, and 81 for the III chromosome. The map of the X given below is in general agreement with the mutation frequency map of MULLER, although independently and somewhat differently derived. When larger populations of mutations are used in each chromosome (populations which have not been selected as probable point mutations), and maps drawn from these, only minor differences from the map here given are observed. In addition samples based on other criteria, for example lethals, Minutes, etc., also give essentially the same map. The samples used in this paper may therefore be regarded as representative and trustworthy. (For further discussion on this point see MULLER and PAINTER 1932.)

These maps are interpreted as giving the spatial distribution of the genes in the chromosomes relative to each other more accurately than at present available from any other source (for comparison with salivary maps, see below). It is not known whether these distances are uniform at all stages in the chromosome and life cycle. The stage of primary interest to the geneticist is that immediately preceding the occurrence of crossing over, and it is to this stage that the interpretation of distance is referred. In the absence of direct measurements at that particular stage, the best criterion whereby one may judge as to whether the maps do reflect the spatial relations at the time of or just before crossing over occurs, is by

examining the cogency of the relations obtaining when the above interpretation is given.

The analyses in the following pages are considered adequate in justifying this interpretation, and accordingly, the maps will be referred to as *leptotene* maps in order to characterize them as simply as possible.

The leptotene maps shown in table 1 were prepared by arranging all reported genes in each chromosome in the order that their reported locations require, and converting the total number of loci in each chromosome to a value of 100 percent. For example, the position on the leptotene map of the gene cut is at 50 on the X chromosome, which means that 50 percent of all the genes reported for the X chromosome lie to the left of cut. Likewise, placing the locus of the gene Stubble at 58.3 in III means that 58.3 percent of all the genes reported in the III chromosome lie to the left of Stubble.

TABLE 1

Leptotene maps of the three major chromosomes.

X		II		III	
LOCUS	GENE	LOCUS	GENE	LOCUS	GENE
0.0	yellow	0.0	aristaless	0.0	roughoid
22.4	white	4.1	Star	5.2	Crimped
24.5	facet	5.1	abrupt	12.5	sepia
29.6	echinus	11.2	dumpy	14.6	hairy
31.6	ruby	16.3	Streak	17.7	rose
37.8	crossveinless	18.4	Bristled	21.9	Minute-h
50.0	cut	23.5	dachs	25.0	Dicheate
59.3	lozenge	25.5	Skin	29.2	thread
62.3	vermilion	30.6	Jammed	32.4	scarlet
66.3	miniature	40.8	black	42.7	Deformed
75.5	sable	50.0	purple	44.8	pink
77.5	garnet	51.0	Bristle	50.0	maroon
88.7	rudimentary	60.1	cinnabar	51.1	curled
90.8	forked	63.3	vestigial	58.3	Stubble
92.8	Bar	67.3	Lobe	60.5	spineless
98.0	fused	73.5	curved	65.7	stripe
100.0	carnation	77.6	lance	74.0	Delta
		81.7	humpy	76.1	Hairless
		82.7	arc	77.2	ebony
		83.7	plexus	80.3	cardinal
		85.8	brown	89.6	rough
		91.9	speck	92.7	Pointed
		100.0	balloon	95.8	claret
				96.8	Minute
				100.0	Minute-g

It will be noticed that the leptotene map of the X chromosome ends with carnation at 100.0. This was done despite the fact that several genes are known to the right of carnation, because most of the chromosome be-

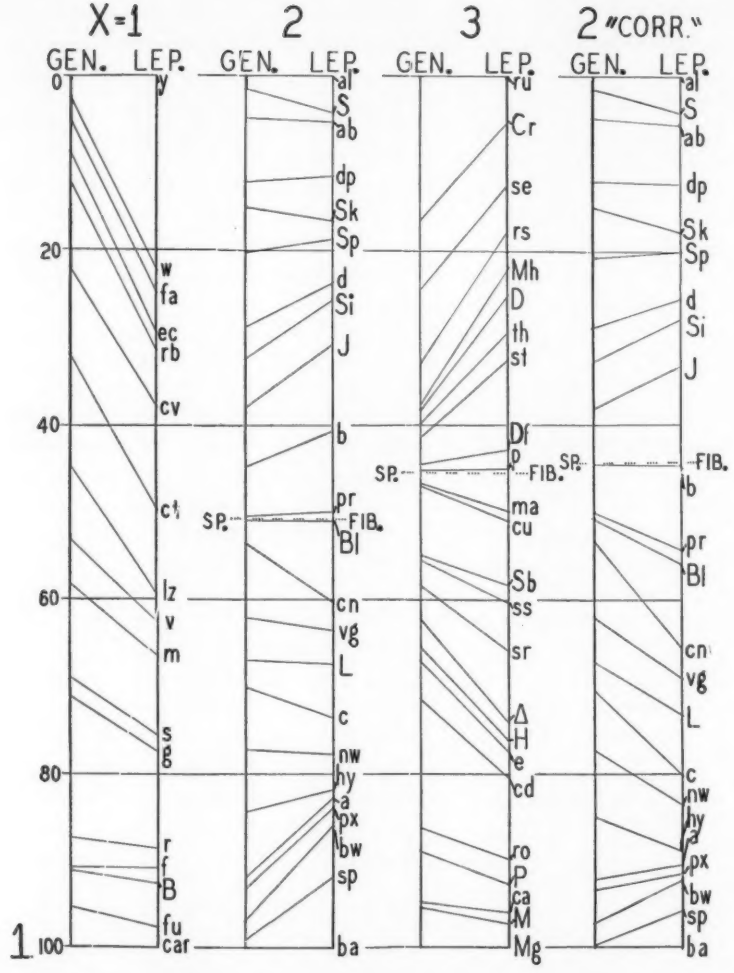

FIGURE 1.—Point by point comparison of genetic and leptotene maps of the three major chromosomes. Genetic maps here, and throughout this paper have been converted to 100 per cent. In II and III, where the spindle fibre insertion point is approximately median, the symmetry is about a corresponding point. In the X, with terminal insertion, the relation of the two maps is comparable to that of one arm of an autosome. The exceptional regions, y–w in the X, and a–ba in II are discussed in the text. The fourth pair of maps are drawn for II, with half the loci between arc and balloon excluded from the leptotene map, as indicated in the text.

yond carnation is inert genetically (MULLER and PAINTER 1932, DOBZHANSKY 1933). For purposes of discussion, it is more convenient to have the chromosome end at carnation because information available from several sources makes it probable that the inert region differs from the active part of the chromosome in its crossing over relations. Actually, however, if the leptotene map is drawn so as to include all genes up to bobbed, the gene at the extreme right end of the X chromosome, the calculations to which the leptotene map is put will not be materially affected. (On my map the locus of bobbed is 107.1).

Figure 1 shows a point by point comparison between the genetic (recombination) and leptotene maps of the three major chromosomes. Several things are apparent from a study of this figure. There is a noticeable regularity in the variation of the leptotene from the genetic maps as the distance from the spindle fibre changes. Chromosome III, for example, shows a symmetry almost exactly about the locus of pink which is the position for the spindle fibre reported by other investigators. Similarly in chromosome II, the symmetry is about a point between purple and Bristle, which also corresponds to the spindle fibre insertion previously reported. The X chromosome is asymmetrical with respect to the genetic map and varies from the rightmost end, which is likewise the end generally accepted, if not proved, as the position of the spindle fibre insertion.

In the leptotene maps, two regions seem to be aberrant, the leftmost region of the X between yellow and white, and the rightmost region of the II beyond humpy. The yellow-white region genetically giving only 1.5 percent of recombination, has a leptotene map distance of 22.4 units. There is a similar crowding of genes to the right of arc. It was thought that the crowding between arc and speck might perhaps reflect the fact that special efforts were made to retain all genes discovered in that region because of the interest in the "pale" translocation, which involved that portion of the chromosome. In an attempt to test this hypothesis, half of the loci between arc and balloon were arbitrarily excluded, and the remaining loci in the II chromosome were used to construct a "corrected" leptotene map. This is also included in figure 1. However, the results of this correction are unsatisfactory because in the first place, the crowding at the right end is not much improved by this treatment, and secondly, the regions in the center of the chromosome are shifted out of place so that the apparent locus of the spindle fibre becomes black, which is unlikely, judging from other data. It is concluded, therefore, that the exceptional regions at the right end of the II chromosome and at the left end of the X chromosome reflect some special conditions in these regions.

This test of the right end of II also indicates that whatever selection has been exercised in the reporting of new loci has not been such as to materi-

ally lessen the statistically representative character of the total loci available.

Changes in details of the leptotene maps will become necessary as further loci become available and are incorporated in them. Experience with these maps for the past year or more has shown that an increase of 10 percent in the number of loci used involves only small corrections, usually less than 1 percent. The error of any locus by this method is probably less than 3 percent.

To facilitate discussion, details of the computation in the rest of the paper have usually been omitted. A sample work sheet is included in the Appendix and the method of computation for the various tables and figures is briefly indicated.

Comparison of results obtained by using leptotene and genetic maps

When curves are drawn, figure 2, showing the change in fraction realized with distance in terms of the leptotene map for data on the completely marked X chromosome, it is found that interference progressively diminishes and then disappears about half the length of the chromosome. These results are sensibly uniform for all portions of the (active) chromosome, and they are practically unchanged when the distances are calculated in terms of ordinary genetic map units as given in figure 3. For the II chromosome, however, the results of these two types of plotting are not equivalent. In figure 4, the change in fraction realized with changing length of segments exchanged, in genetic units, is plotted. It will be seen that the results are variable. Especially in region 4, purple-cinnabar, which is in the vicinity of the spindle fibre, is the deviation most pronounced. When for the same data fraction realized is plotted against leptotene lengths exchanged, however, these variations practically disappear. In figure 5 regions 3, 4, 5, 6 and 7 are all on essentially one curve. *In this case the spindle fibre region falls completely in line with the results in the other regions.* It will be seen in this figure that the curves for regions 1 and 2 do not coincide with the curves for the other regions. It is considered that this remaining variation is due to the fact that there are undetected doubles within region 2 (dumpy-black) for which no corrections in the data have been made, and therefore regions to the left of it will have their results modified. This conclusion is supported by comparison of the curves for region 7, which, in figure 4, coincides with region 2, and falls on the average curve in the leptotene plot. It is to be expected that similar analyses of more comprehensive data in the same chromosome would yield still more uniform results. The similarity of the leptotene and genetic plots of the X is consistent with this finding for the II because of the terminal position of the spindle fibre in the X and the presence of a large amount of inert chromatin between it and carnation, which is the rightmost gene used.

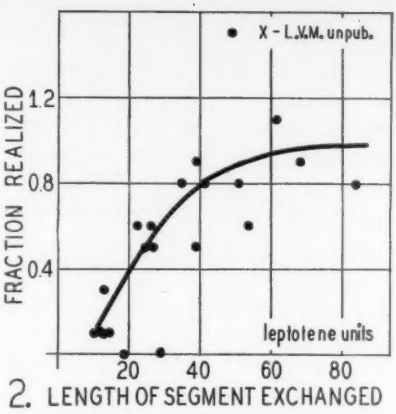

FIGURE 2.—Change in interference with progressive increase in the size of segment exchanged for the X chromosome, in units of the leptotene map. Separate curves for each region fit the average curve. Compare with figure 6. Data of L. V. MORGAN unpublished, 3,570 flies.

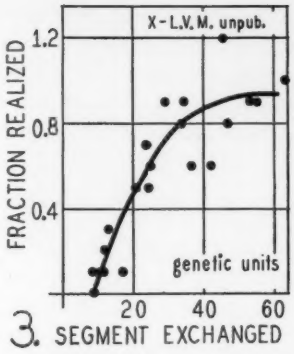

FIGURE 3.—Same as figure 2 except that distances (abscissa) are measured in ordinary genetic units. The curve is similar to that of figure 2.

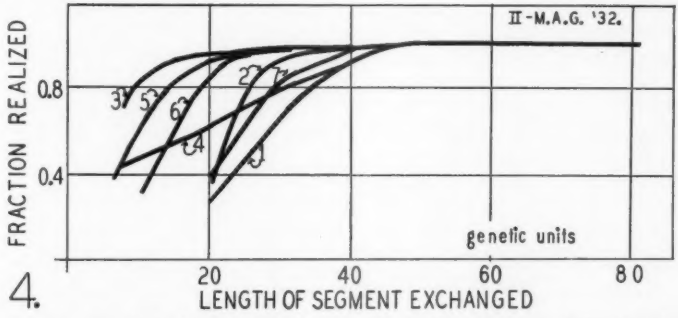

FIGURE 4.—Correlation between size of exchanged segment, in genetic units, and interference relations for the II chromosome. The curve for each region is the average of 28 points. The curves differ among themselves, especially region 4. Data of M. A. GRAUBARD 1932, 19,058 flies.

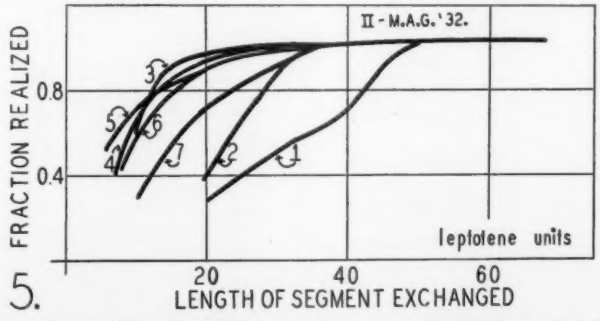

FIGURE 5.—Same as figure 4 except that segment length is measured in leptotene units. The curves correspond, except for regions 1 and 2, which are discussed in the text.

It is necessary to emphasize the necessity of using data on completely marked chromosomes in making analyses which will directly reflect the cytological events. The maximum amount of undetected doubles should not exceed 0.5 percent in any region and should be less than 1 percent for the entire chromosome (SCHWEITZER 1934, and unpublished calculations). This can be illustrated by a consideration of the data of BRIDGES and OLBRYCHT using alternated X-ple ($\frac{y\ cv\ v\ f}{ec\ ct\ g}$). In plotting these data similar to figure 2, fraction realized against leptotene length, figure 6, it appears that the cut-vermilion region is aberrant in that it does not show the same rise in fraction realized as is shown by the other regions. When it is realized, however, that the data do not go beyond the locus of forked, it becomes clear that it is impossible for the cut-vermilion region which is in the middle of the chromosome to give interference relations comparable to the other regions because unless the chromosome is marked to the rightmost end, there will be no region that will be independent of the cut-vermilion region in the experiment, and the fraction realized will consequently never rise to one. When the data of L. V. MORGAN on the more completely marked chromosome ($\frac{y^2\ cv\ v\ f}{y\ ec\ ct^6\ s\ car}$) mentioned above are plotted however (figure 2), this anomalous result disappears. It is seen therefore that the use of the leptotene map has simplified the analysis by

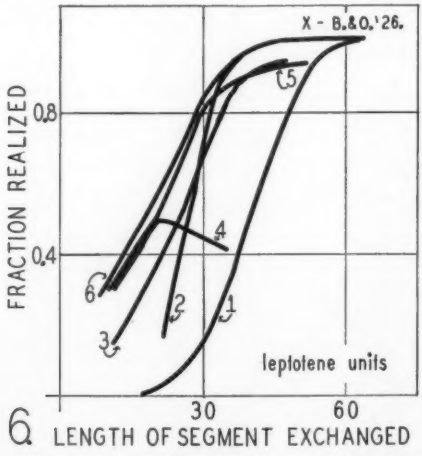

FIGURE 6.—Same plot as figure 2 from data of BRIDGES and OLBRYCHT, 20,786 flies. In this case, region 4 (cut-vermilion) does not fit the average curve for the other regions. As explained in the text, this is due to the fact that the region to the right of forked was not marked in the experiment.

eliminating the spindle fibre as an influence in interference relations. Interference relations between regions are determined *only* by the physical distances between them.

Another practical demonstration of the greater effectiveness of the leptotene map as the basis for analyzing crossing over relations comes from the study of the effect of temperature on size of internode or length of section exchanged in a double crossover.

The recovery of gametes having such combinations of genes as make it possible to judge that two regions of interchange were involved in a single chromatid, makes it possible to estimate the internodal length. Referring again to the previous example, a 1, 4 double crossover would have a leptotene length equal to $1/2\ (1+4)+2+3$. This measure is obtained by plotting the frequency of double crossovers against the length of the internodal segment, and may be made for all regions of the chromosome. When such a plot is made for the normal II chromosome, at various temperatures between 14° and 30°, figure 7, the results obtained are sensibly uniform at all temperatures. When the plot is made using recombination percent as the abscissa, however, no general correlation can be made for the results at different temperatures, as can be seen in figure 8. Here again is seen the tremendously simplifying relationship obtained by using the leptotene plot. The temperature effect will be referred to again in the following pages.

These practical advantages would alone justify the use of the leptotene maps as the basis for analyzing the crossing over data and accepting them as representative of the spatial distribution at the time of crossing over. There is however in addition a theoretical advantage to be derived from the use of these maps. The recombination data as summarized in the genetic maps give the results of the operation of the forces involved in synapsis, exchange and disjunction, taken together. And it is impossible to analyze the components possibly contributed by each of these three forces to the end results, namely, recombination of linked genes. Since the leptotene maps are taken to give the location of the various genes in space in the chromosomes, they make possible a study of the mechanism of exchange by itself, because with their use, a study of this one variable alone may be made.

ANALYSIS OF CROSSING OVER IN THE NORMAL CHROMOSOMES

It was consistently found during the course of the calculations that only data involving good and equally viable contrary classes and involving fairly large numbers covering chromosomes completely marked, gave clear-cut results when analyzed, as here indicated. Consequently, it was not possible to check up each conclusion from one set of data with all the other extant data that might otherwise be relevant. The general principle

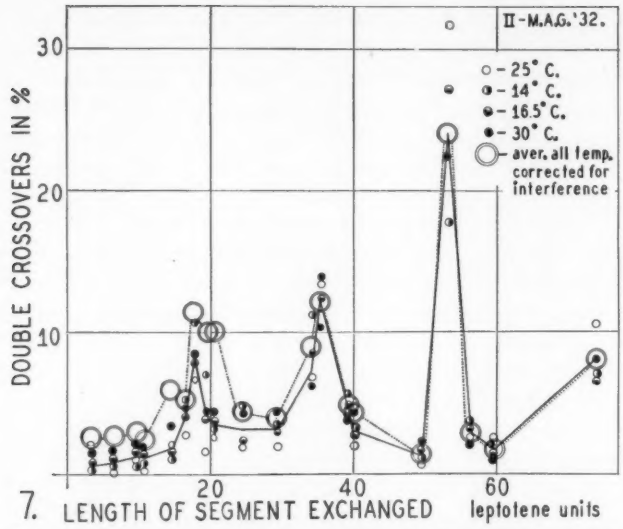

FIGURE 7.—The frequency of double crossover classes of different leptotene lengths, at four temperatures for the II chromosome. The average curve clearly fits the data at all temperatures. The large dotted circles are the average for all temperatures, each corrected for its own interference value. It is apparent that there are no important differences between the two curves. Data of M. A. GRAUBARD.

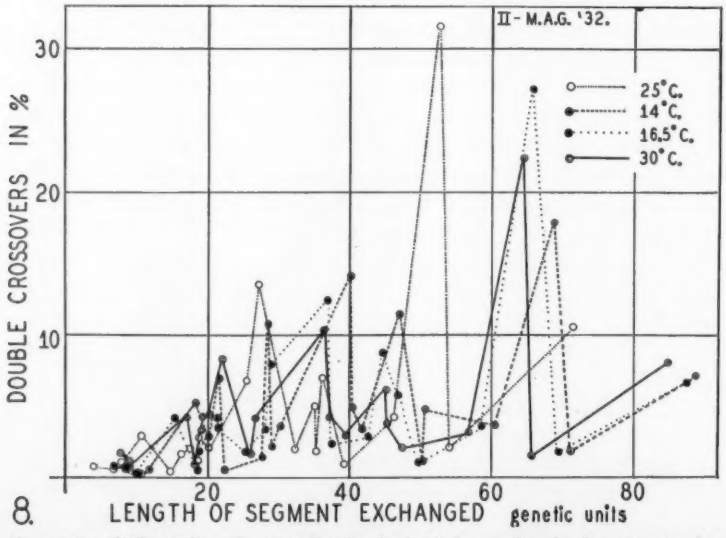

FIGURE 8.—Similar to figure 7 except that the abscissa is in genetic units. In contrast to figure 7, no single curve can be drawn. For example, at lengths 40 and 50, the different temperatures have widely different frequencies of doubles, and the curves for several temperatures are going in contrary directions.

has been followed, however, in placing most credence on the results obtained from what seemed to be the best data, judging by the criteria indicated above. However, every case of important or substantial disagreement between the results, obtained from analyzing different sets of data, has been indicated.

The X chromosome

For the study of crossing over in the X chromosome, the following data were used: X-ple, BRIDGES and OLBRYCHT 1926 (20,786 flies) and the unpublished data of L. V. MORGAN referred to above (3,570 flies). In

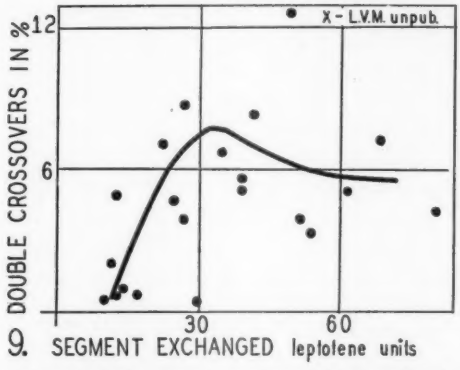

FIGURE 9.—Frequency of double crossovers for different leptotene lengths of the X. This curve appears modal at about 30 units. Data of L. V. MORGAN.

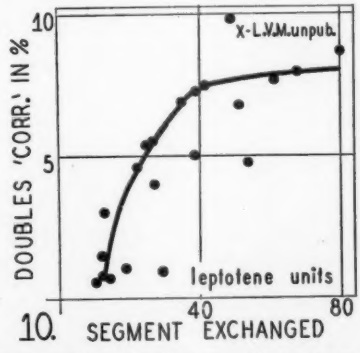

FIGURE 10.—The same plot as figure 9 with a correction factor applied to the frequency of doubles in each class, to correct for the inequalities in length of chromosome between the loci used in the experiment. There is no modality in this figure.

addition, some calculations were made on the control data of L. V. MORGAN, 1933 (*ec-bb*, 2,623 flies).

Interference progressively disappears as the distance from the region of first interchange increases, becoming negligible beyond about half the total length of the X. This has already been demonstrated (SCHWEITZER loc. cit. tables 1, 2 and 5) and in figures 2, 3 and 6 above.

When double crossovers are plotted against leptotene length, as in figure 9, the results might be interpreted as demonstrating the presence of a modal length for the internodal segment. That this mode is spurious rather than real is shown when the same plot is made except that each region is corrected for the frequency of expected crossing over within the regions involved. This correction is necessary because the loci used in the experiment are not equidistant from each other. For example, scute-echinus is 7.5 units, cut-vermilion is 13.0 units. With the same frequency of occurrence of crossing over, there should be 1.7 times as many crossovers in the cut-vermilion region as occur in the scute-echinus region. The correction is made by multiplying each frequency by an appropriate factor derived from the expected amount of crossing over involved in each of the two regions due to this factor. Figure 10, which has been derived in this way, shows that the frequency of double crossovers increases as interference lessens and remains constant for all distances not involving interference. If now of the corrected doubles, those involving interference are

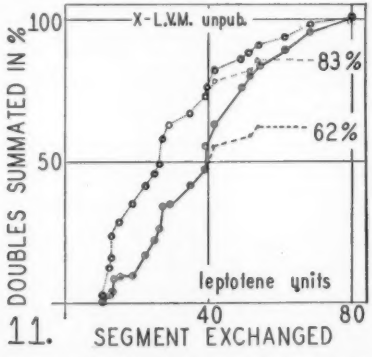

FIGURE 11.—Proportion of doubles that involves interference in the X. In the lower curve the doubles are corrected as in figure 10, and summated in order of length, and the doubles involving interference are also summated separately. In the upper curve, the data is also corrected for interference before summated. Sixty-two per cent of all doubles that occur involve interference. Eighty-three per cent of those that would occur involve interference. Data of L. V. MORGAN.

separated from the rest and summated for increasing distance and compared with the total number of corrected doubles, as in the lower curve of figure 11, the indication is that about 2/3 (62 percent) of all nodes that form (measured genetically as double crossovers) involve interference. The upper curve in the same figure, which has also been corrected for inter-

ference at every point, indicates that about 4/5 (82 percent) of all nodes that attempt to form (measured by interference) do in fact interfere with each other.

That the results are uniform for all regions may be shown by comparing the frequency of double crossovers for each region separately. These have not been reproduced but all give a fit to the average curve.

Another measure of the effectiveness of interference in different parts of the chromosome may be made by studying the size of independent regions in various parts of the chromosome. This is obtained by interpolating for each region of the map the leptotene length that must be included before interference just disappears. Table 2, which includes the values from all available data, indicates that this is sensibly constant throughout the chromosome length.

TABLE 2

Length of intervening segment, leptotene units, from a region where a crossover has occurred to the region where another occurs without interference due to the first.

REGION	LOCUS OF MIDPOINT	LENGTH OF SEGMENT (INTERPOLATED)			AVERAGE
		B. & O. 1926	L.V.M 1933	L.V.M. UNPUB.	
y-ec	15			70	70
sc-ec	15	65			
ec-cv	34	50	50	55	50
cv-ct	44	50	40	60	50
ct-v	56	60	60	50	55
v-s	69			50	50
v-g	70	60	45		
s-f	84			55	50
g-f	83	50	40		
f-car	95			50	50
f-bb	99		50		

A few words should be said on the meaning of the term "loop length." This term, as commonly defined and used, refers to the internodal length. It has been used to refer to the modal length of internodal segment. But in view of the absence of modal loop lengths, it is necessary to revise the terminology. In this paper, the term "loop length" is not used; the more general term "internodal length" refers to segments of interchange involved in a double crossover (BRIDGES 1915, 1929).

The II chromosome

For this chromosome, the data of GRAUBARD 1932 and of REDFIELD 1932 have been analyzed. In REDFIELD'S data, the contrary classes are unequal in many cases, and this, in addition to the fairly small numbers involved,

sometimes led to irregularities in the results when plotted. In all cases, however, where comparable figures could be drawn, the results were in agreement with those obtained from GRAUBARD'S data.

The data on fraction realized have already been discussed in connection with the leptotene maps (figures 4 and 5), and also in connection with the use of fraction realized as a measure of interference (see SCHWEITZER loc. cit. tables 3 and 6).

The plotting of internodal lengths in figure 7 seems to show the existence of a mode. This mode, like the one in the X chromosome (figure 9) is

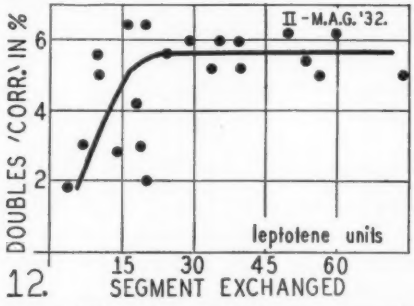

FIGURE 12.—Frequency of double crossovers of different sizes corrected for non-equidistance of the intervals between the marked loci for the II chromosome. This figure is inconsistent with the interpretation of modal length of segments in double crossing over. Data of M. A. GRAUBARD.

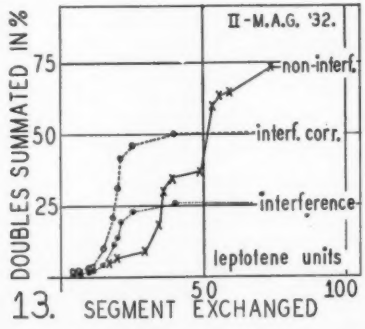

FIGURE 13.—Interference and non-interference double crossovers in the II chromosome. Twenty-five per cent of all doubles involve interference. An additional 25 per cent fail to appear due to interference. Data of M. A. GRAUBARD.

spurious, however, for when the data are corrected in the manner indicated for the X chromosome, this supposed modal length also disappears. Figure 12 shows clearly that for the II chromosome there is no modal distance between the two breaks in double crossing over. Separate plots for each region, not included here, show that in this chromosome, as in the

X, the internodal lengths are similar in distribution for all regions of the chromosome.

It has already been pointed out that the two arms behave like two independent chromsomes. It might perhaps therefore be expected that analyses of data in each arm taken separately would yield different results, but this does not turn out to be the case. The internodal lengths within a single arm have a modal value only if the data are not corrected for the expectancy of crossing over in the various regions (curves not given).

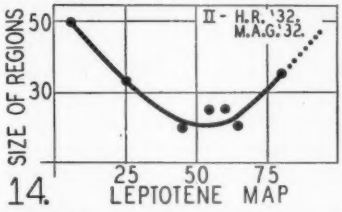

FIGURE 14.—Size of regions giving no interference in double crossing over in the II chromosome. The curve may be interpreted as symmetrical about the spindle fibre. Data of H. REDFIELD (1932) and M. A. GRAUBARD (1932).

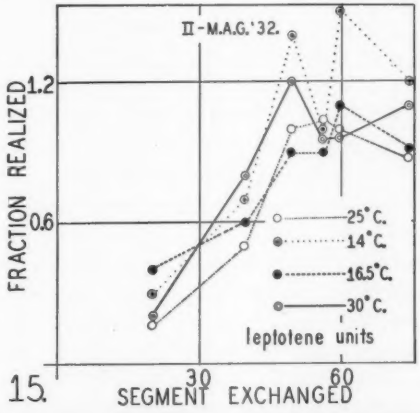

FIGURE 15.—Effect of temperature on fraction realized in the II chromosome. There is no temperature effect on interference. Data of M. A. GRAUBARD.

It is possible also in this chromosome to estimate the frequency of interference loops. It turns out in this case, figure 13, that about 25 percent of all recovered internodal segments show interference, and an equal number fail to appear due to the effect of interference.

It is possible also to study directly the effect of interference upon internodal length. Correction of graphs such as figure 7 for interference

shows, large dotted circles in figure 7, that interference does not affect the frequency distribution of internodes.

For the II chromosome, the size of independent regions is not constant throughout the chromosome, as was the case in the X chromosome. Figure 14 indicates that the size of independent regions varies symmetrically about the spindle fibre.

The III chromosome

For this chromosome, there was no single set of good data available for study. The curves of the analyses represent, in part, studies from data of BRIDGES (1929), in which the region to the right of sooty was not marked, several sets of data by DOBZHANSKY (1930, 1932) which were published as controls for various translocation experiments, and the data on normal chromosomes published as controls by REDFIELD (1930) and BEADLE (1932) summarized together. Only in the case of BRIDGES, were the contrary classes fairly equal. The other data had to be used, however, since in BRIDGES' experiment a large portion of the right limb of the chromosome was unmarked. Since the curves for this chromosome are all similar to the preceding ones, none have been reproduced.

The plot of fraction realized for all three sets of data is in general similar to that of GRAUBARD on the II chromosome. A characteristic of all three curves is that the fraction realized regularly rises to values greater than 1.0. This may be interpreted as being due: (a) to the larger size of the III chromosome (it is reported to be 1/3 larger than the II cytologically) and (b) to the presence in it of a larger average number of chiasmata than occur in the II chromosome (MATHER 1933, having shown from the analysis of diploid data that the average number of crossovers is 1.94 in II and 2.4 in III).

It might be expected from this that a modal length would be found in this chromosome. A plot of corrected internodal length similar to those for X and II from REDFIELD'S and BEADLE'S and DOBZHANSKY'S data shows the absence of modal values. BRIDGES' data give an apparent modality but his data is unsuitable for this type of calculation because of the presence of a long terminal unmarked region, as was shown in the discussion of the X-ple data (figure 6 above). As in the case of the II chromosome, when non-interference internodes are plotted separately, there is no indication of modality. More detailed analyses of each single region in these data make the conclusion inescapable that there is no modal length because each separate region gives an approximate fit to the curve for the data as a whole. Such figures also show that there are no regional differences throughout the length of the III chromosome.

Since the best data for this chromosome do not cover the entire map,

no analysis of the size of independent regions in all parts of this chromosome can be made.

VARIATIONS IN CROSSING OVER

Temperature

The effect of temperature on crossing over has been studied by several observers, PLOUGH (1917, 1921), STERN (1926) and GRAUBARD (1932). Only in GRAUBARD's data, are available variations in crossing over in chromosomes completely marked.

Temperature changes above and below 25° increase the crossover values in the II chromosome near the spindle fibre, but not in the more distal regions. Because of the regional differences, changes in internodal length and in interference relations may be profitably studied for this data, to determine the influence of crossing over on internodal length, interference, etc. Some of the results have already been indicated.

As far as has been determined, temperature has no effect whatever on

TABLE 3

Fraction realized and proportion of double crossovers for all temperatures in II.

CLASSES	3A. FRACTION REALIZED					3B. PROPORTION OF DOUBLE CROSSOVERS				
	25°	14°	16.5°	30°	AVERAGE	25°	14°	16.5°	30°	AVERAGE
1,2	0.2	0.3	0.4	0.2	0.28	2.8	2.8	3.4	4.4	3.4
1,3	0.5	0.7	0.6	0.8	0.65	1.8	3.3	2.7	2.9	2.7
1,4	1.0	1.4	0.9	1.2	1.13	0.9	1.1	1.0	1.9	1.2
1,5	1.0	1.0	0.9	1.0	0.98	4.1	3.1	3.5	3.1	3.5
1,6	0.9	1.5	1.1	1.0	1.13	2.0	1.8	1.8	1.5	1.8
1,7	1.1	1.2	0.9	1.1	1.08	10.6	7.0	6.7	8.2	8.1
2,3	0.2	0.4	0.5	0.5	0.40	1.6	7.0	4.2	4.3	4.3
2,4	0.8	1.0	1.1	0.5	0.85	2.0	3.5	3.2	4.3	4.3
2,5	1.2	1.0	1.0	0.9	1.03	13.4	14.0	12.3	10.3	12.5
2,6	0.9	0.9	1.1	1.0	0.98	4.9	4.7	5.7	3.8	4.8
2,7	1.1	0.9	1.0	1.1	1.03	31.7	17.8	27.2	22.4	24.8
3,4	0.7	0.2	1.8	0.4	0.78	0.7	0.5	0.8	1.6	0.9
3,5	1.1	0.8	0.8	1.1	0.95	2.8	5.2	4.1	4.3	4.1
3,6	1.0	0.9	1.0	0.9	0.95	1.2	2.1	1.7	1.6	1.7
3,7	1.0	1.0	0.9	0.8	0.93	6.9	11.3	8.6	6.4	8.5
4,5	0.4	0.3	0.4	0.5	0.40	0.5	0.5	0.6	1.1	0.7
4,6	0.5	0.7	0.2	1.0	0.60	0.3	0.5	0.4	0.9	0.5
4,7	0.7	0.8	0.7	0.8	0.75	1.9	4.8	2.3	4.4	3.4
5,6	0.4	0.6	0.8	0.6	0.60	1.0	0.1	0.3	0.9	0.6
5,7	0.6	0.8	0.6	0.7	0.68	6.7	10.7	7.9	8.2	8.4
6,7	0.4	0.1	0.3	0.6	0.35	2.0	1.4	1.8	3.3	2.1

the general relationships. In table 3a, the average value of fraction realized for each class at all temperatures, is indistinguishable from the results obtained at 25°. Table 3a gives the fraction realized of each internodal segment for each temperature. This shows that the interference relations are unchanged. Figure 15, which is a detailed comparison at each temperature of region 1, and is representative of conditions throughout the chromosome, shows further that there is no constant effect associated with any particular temperature, indicating that the mechanism is unaffected. Table 3b shows that the proportion of internodes is closely comparable for all temperatures, and that in this case also no systematic differences occur. There is likewise no modal length of internode at any temperature.

Age

Variations in crossing over in relation to the age of female was first reported by BRIDGES in 1915 on the II chromosome. Since that time, studies of the effect on each of the chromosomes have been made by PLOUGH (1917, 1921), STERN (1926), BRIDGES (1927, 1929), BERGNER (1928). The only data on the age effect which is sufficiently comprehensive to be useful for analyzing the results on the general mechanism of crossing over is that of BRIDGES on the III chromosome. In this experiment, seven loci were simultaneously followed covering all of the left arm and $\frac{1}{2}$ of the right arm, up to sooty (e^s). Females were transferred to fresh food every two days, so that it is possible to compare the results at close intervals. BRIDGES found that crossing over was decreased from an original maximum in the first three days to a minimum at about the ninth or eleventh day, an average of 36 percent for the entire chromosome, with relatively unimportant changes thereafter. He also reported changes in coincidence relations with age. As in the case of temperature, variations in crossing over with age are not uniform for all parts of the chromosome.

A study of the interference changes during the early days of egg-laying, when the crossing over changes are most pronounced, reveals no consistent changes with age. Computations were made for many combinations of egg-laying periods. Three kinds of calculations are given in table 4 for each of ten egg-laying periods, which would be expected to show the widest differences among themselves.

In table 4a, the values of fraction realized for each of these ten egg-laying periods are quite comparable to each other and to the results of the normal period (0–11 days), which has been discussed in connection with the analysis of the III chromosome.

The corrected frequency of double crossovers for these egg-laying periods given in table 4b are quite comparable in all respects to similar calculations on the standard III chromosome. The apparent modality is considered spurious for the reasons previously mentioned.

TABLE 4

Fraction realized, corrected doubles and length of independent regions for various egg-laying periods in III.

		EGG-LAYING PERIOD									
	CLASSES	0–39	0–31	0–21	0–11	0–5	5–11	11–17	11–21	21–31	31–39
4A. FRACTION REALIZED	1, 2	0.2	0.2	0.2	0.2	0.3	0.6	0.7	0.3	0.2	*
	1, 3	0.1	0.8	0.8	1.3	0.9	0.9	1.0	0.7	0.6	0.0
	1, 4	1.1	1.4	1.4	1.7	1.9	1.0	0.9	0.7	0.5	*
	1, 5	1.3	1.3	1.3	1.5	1.8	1.2	1.1	1.0	0.2	*
	1, 6	1.5	1.5	1.5	1.3	1.5	1.2	1.7	1.7	1.2	2.4
	2, 3	0.1	0.2	0.2	0.3	0.2	0.3	0.0	0.0	0.0	0.0
	2, 4	0.7	0.5	0.5	0.7	0.6	0.6	0.3	*	1.0	*
	2, 5	1.1	1.2	1.2	1.0	1.2	1.2	*	0.9	0.9	1.8
	2, 6	1.3	1.2	1.2	0.9	1.0	1.1	1.4	1.6	1.1	1.1
	3, 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3, 5	1.6	1.5	1.3	1.1	0.3	3.0	*	*	*	*
	3, 6	0.7	0.7	0.9	0.7	0.3	1.4	0.0	0.9	0.0	0.0
	4, 5	1.5	1.5	1.7	1.7	1.1	2.8	*	0.8	*	0.0
	4, 6	1.0	1.0	0.9	1.1	1.3	0.3	*	*	1.1	1.6
	5, 6	1.6	0.4	0.3	0.3	0.3	0.4	0.3	2.5	0.6	0.0
4B. CORRECTED DOUBLES	1, 2	3.2	3.3	3.2	2.9	2.1	3.3	3.6	4.0	4.5	*
	1, 3	10.0	8.0	6.6	7.3	6.2	10.9	1.5	0.7	*	0
	1, 4	18.0	12.5	13.2	12.2	8.9	16.1	14.3	10.8	7.5	*
	1, 5	18.0	12.2	12.5	10.4	8.0	13.1	22.4	13.0	18.6	*
	1, 6	13.0	12.4	12.1	9.6	6.8	13.9	20.0	15.4	17.0	1.6
	2, 3	4.7	4.0	5.0	6.2	1.6	11.2	0	0	0	12.9
	2, 4	14.3	7.5	7.0	7.0	4.1	13.0	*	*	2.4	*
	2, 5	20.4	13.0	13.0	11.8	8.0	18.0	15.2	16.3	18.0	13.5
	2, 6	14.7	12.9	12.0	9.3	5.5		18.1	17.1	20.0	6.4
	3, 4	1.4	1.0	5.0	5.7	0.0		0	0	0	*
	3, 5	13.0	14.0	18.6	12.6	*		*	*	*	36.2
	3, 6	4.3	10.0	13.0	8.1	*		0	0.8	0	0
	4, 5	17.2	20.0	22.0	14.5	5.7		*	13.0	*	20.1
	4, 6	8.9	11.5	11.0	10.0	7.4		*	*	1.5	1.7
	5, 6	3.9	4.9	5.0	4.1	3.2		6.7	5.2	7.5	4.3
4C. LENGTH OF INDEPENDENT REGION	Region										
	1	4	4	4	3	4	4	3	5	6	*
	2	5	5	5	5	5	5	6	6	4	5
	3	5	5	5	5	7	6	*	*	*	*
	4	5	5	5	5	5	5	*	*	6	6
	5	4	4	4	4	4	4	*	*	*	*
	6	5	4	2	4	4	3	2	5	4	4

* Numbers too few to be significant.

Calculation of the size of independent regions for these different egg-laying periods reveals no apparent change with age (table 4c).

DISCUSSION

The role of the spindle fibre region in crossing over has been clarified. The spindle fibre separates the autosomes of Drosophila into two units with independent genetic behavior. This conclusion is consistent with the observations on the salivary gland recently reported by PAINTER (1933, 1934), in which the arms of the autosomes appear physically independent of each other. It may be compared with the condition found in other insects (summarized in WILSON 1925), where independent chromosomes sometimes become attached during the meiotic stages. In this case, the attachment is persistent in germinal tissues, and the separation, when it occurs, takes place late in embryogeny. The spindle fibre has no specific effect on the process of exchange. Regions in its immediate neighborhood behave in all respects as do regions further removed.

If exchange usually starts at some definite point, it might be expected that the cytological lengths of chromatin showing interference would be changed when crossing over is changed. The absence of any effect of age and temperature on interference, when both are known to change the recombination values, and have a differential effect in different regions of the chromosomes, would seem to indicate that there is no definite region where crossing over first occurs and from which the frequency of crossing over in other regions is influenced. Crossing over after pairing occurs independently (and perhaps simultaneously) throughout the length of the chromosome.

Many of the problems associated with interference have already been discussed. Interference is revealed as a local phenomenon associated with regional events but with no central determining mechanism. Interference therefore need not necessarily be interpreted as a mechanical factor affecting the chance of an exchange at any locus; it may be a reflection of the mathematical generalization that in a chromosome having only a limited number of crossovers, relatively few of them will occur close together. This interpretation, if supported, would negate most of the current hypotheses regarding the mechanism of interference as due to twisting of the chromosome, breakage, etc. The published data on inversions (GRAUBARD 1932, 1934) support this conclusion, and preliminary calculations of interference and the cytological properties of inverted and translocated chromosomes give results wholly consistent with this interpretation. But at present, the evidence is insufficient to decide between these alternatives. In triploids, the published data make it probable that change of partners occurs with sufficient frequency so that interference is very much

decreased, and it is to be expected therefore that calculations from the data on crossing over in triploids, when corrected as indicated in this paper, will give recombination percentages for all regions equivalent to those appearing in the leptotene maps.

The phenomenon of recombination, as already mentioned, reflects the operation of forces of pairing, exchange and disjunction. Similarly, theories of crossing over require analyses and hypotheses regarding the interaction of these three kinds of forces. The material presented in this paper is concerned primarily with the forces of exchange, including the location of the genes at the time of exchange, the formation of overlaps and the internodes between succeeding overlaps, and in addition, the problem of interference. Pending the conclusion of analyses involving situations in which the chromosomal configuration has been changed, translocations and inversions, where the forces of attraction (pairing) are affected, and of the data on non-disjunction where the forces of disjunction are interfered with, it is premature to attempt to pass judgment on current hypotheses regarding the mechanism of crossing over. All theories of crossing over now current are inadequate in their analysis of interference.

If the recently described salivary chromosomes represent the normal distribution of genes in all tissues, it will be possible to make analyses equivalent to those here presented from salivary maps as soon as enough loci are identified. At present, the most detailed salivary map is of the X. It is consistent with the leptotent map and yields similar results on analysis. This cannot be taken as verification of the leptotene maps, however, since the leptotene map of the X is not very different from the genetic map. A more critical comparison will be possible when detailed salivary maps of II and III appear.

From the manner of their construction, the leptotene maps exclude the inert regions from consideration. This is inevitable because our information about crossing over is limited to those portions of the chromosomes that have *active* chromatin (by which is commonly meant regions that (a) contain genes which mutate with measureable frequency, and (b) the loss or addition of any considerable portion of which has a marked influence on the viability, somatic characteristics, etc. of the tissue concerned). The localization of large amounts of inert chromatin in the vicinity of the spindle fibre of the three major chromosomes may afford a clue to the effect of age and temperature, whose crossing over effects are also largely localized near the spindle fibre.

A theoretical difficulty common to all analyses of Drosophila data on crossing over, including this one, is that they are based on the recovery of single chromatids while the reference is intended to the tetrad. Until the role of the forces in the tetrad, especially the interrelation between

exchange and disjunction is clarified, this weakness will continue to be important. The simplicity of the relations and the mutual consistency of diverse types of data on all chromosomes as presented in this paper, renders it probable, however, that this and other uncontrolled factors are of secondary importance.

SUMMARY

1. The data and concepts of coincidence and interference are analyzed, and a new method of calculating interference relations is suggested.

2. Interference relations are uniform for the X chromosome, which has a terminal spindle fibre insertion region.

3. Interference relations for the II chromosome and the III chromosome are likewise uniform. These have a median spindle fibre insertion. A discussion of the role of the spindle fibre and of interference is included.

4. New maps of the chromosomes of Drosophila are constructed. These "leptotene" maps are interpreted as giving the distribution of the various loci at the time of crossing over.

5. There is no modal length of internodal segment in X, II or III chromosomes. For regions not involving interference, internodes of all sizes are equally likely to occur.

6. Two-thirds of the crossover chromatids in the X involve interference.

7. Twenty-five percent of the crossover chromatids in the II chromosome involve interference.

8. Interference does not affect the cytological length of internodal segment.

9. Crossing over does not begin at any particular place on the chromosome.

10. Temperature does not influence the values of interference, the frequency of internodal length, or the size of independent regions.

11. Age changes in the female do not affect the values of interference, the frequency of internodal length, or the size of independent regions.

12. It is concluded that temperature and age changes have no effect on the general mechanism of crossing over, but that each has only specific and regional effects on the frequency of occurrence of crossing over.

13. The absence of any general effect of age and temperature justifies the use of a ten-day egg-laying period and 25° as standardized conditions for the acquisition of data for the analysis of the mechanism of crossing over.

APPENDIX

Computations

The tables and graphs have been made directly from work sheets, of which one is given in the accompanying table.

#6A-2 $y\,\underset{1}{}\,cc\,\underset{2}{}\,cv\,\underset{3}{}\,ct\,\underset{4}{}\,v\,\underset{5}{}\,s\,\underset{6}{}\,f\,\underset{7}{}\,car$ X-chromosome, 25°
#=3570 flies
N = total of (d+t) = 410
L.V.M. unpub.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
CLASS	SIZE	GENETIC LENGTH IN PERCENT	STANDARD GENETIC LENGTH IN PERCENT	LEPTOTENE LENGTH	COINCIDENCE	FRACTION REALIZED	$a\times b\times 10^{-2}$	$d+t$	$\frac{\Delta d+t}{N}\times 10^{-2}$	$\frac{d+t}{N}$	DOUBLES $a\times b$ IN PERCENT	COLUMN 11 FR. REAL. IN PERCENT	COLUMN 11 SUMMATED	COLUMN 12 SUMMATED	DOUBLES FR. REAL.
1,2	6	8.2	11.0	18.9	0.1	0.0+	0.65	3	0.7	9.8	1.1	10.4	7.7	32.1	6.9
1,3	11	17.0	22.6	29.1	0.1	0.0+	0.56	2	0.5	34.8	0.8	8.6	27.9	58.1	2.5
1,4	15	29.0	38.0	41.3	0.9	0.8	1.07	34	8.3	63.6	7.4	4.8	54.5	75.7	5.2
1,5	18	42.0	56.4	53.9	0.6	0.6+	0.69	14	3.4	83.6	4.8	3.8	75.7	87.1	4.1
1,6	20	53.6	75.5	68.2	0.9	0.9+	0.90	30	7.3	96.0	7.9	4.3	91.2	94.7	3.6
1,7	21	63.6	91.1	80.4	1.0	0.8+	0.46	17	4.2	100.2	8.7	5.3	99.9	100.0	2.1
2,3	1	8.9	11.6	10.2	0.0	0.1	0.78	2	0.5	0.5	0.6	2.8	0.6	2.8	2.5
2,4	7	20.9	27.0	22.4	0.5	0.6	1.50	29	7.1	16.9	4.5	3.8	12.2	35.9	5.9
2,5	12	33.9	45.4	35.0	0.8	0.8	0.96	28	6.8	41.6	6.9	4.2	34.8	62.3	4.1
2,6	16	45.5	64.5	49.3	1.2	1.5	1.25	52	12.7	76.3	9.7	3.3	64.2	79.0	6.2
2,7	19	55.5	80.1	61.5	0.9	1.1	0.65	21	5.1	88.7	7.6	3.3	83.3	90.4	2.5
3,4	2	12.0	15.4	12.2	0.2	0.1	1.30	8	2.0	2.5	1.5	7.1	2.1	9.9	9.8
3,5	8	25.0	33.8	24.8	0.6	0.5	0.83	19	4.7	21.6	5.4	5.2	17.6	41.1	4.6
3,6	13	36.6	52.9	39.1	0.6	0.5	1.08	23	5.6	47.2	5.0	4.8	39.8	67.1	5.5
3,7	17	46.6	78.5	51.3	0.8	0.8+	0.56	16	3.9	80.2	6.7	4.3	70.9	83.3	2.1
4,5	4	13.0	18.4	12.7	0.3	0.3	1.60	20	4.9	8.1	3.0	4.7	5.9	18.4	8.0
4,6	10	24.6	37.5	27.0	0.5	0.5	2.09	36	8.8	34.3	4.0	3.7	27.1	49.5	8.6
4,7	14	34.6	53.1	39.2	0.9	0.9	1.07	33	8.3	55.3	7.3	3.8	47.1	70.9	4.5
5,6	5	11.7	19.1	14.3	0.1?	0.1	1.33	4	1.0	9.1	0.7	3.3	6.6	21.7	4.9
5,7	9	21.7	34.7	26.5	0.7	0.6	0.69	16	3.9	25.5	5.5	4.7	23.1	45.8	3.2
6,7	3	10.0	15.6	12.3	0.1	0.1	0.90	3	0.7	3.2	0.8	3.8	2.9	13.7	3.4

In Column 1, the classes are arranged in order of size as measured in leptotene lengths. Class 2, 3 is the smallest; 3, 4 next larger, etc.

Column 2 gives the length of each class in genetic units as computed directly from the original data. The position of the breaks is taken to be midway between the loci followed in each case. For class 1, 4 the length is equal to $1/2\ (1+4)+2+3$, which equals $1/2\ (6.8+15.8)+9.5+8.2 = 29.0$. The total map is made equal to 100 units.

Column 3 gives the length of each class in standard genetic units from the latest published maps of BRIDGES (in T. H. MORGAN, The Scientific Basis of Evolution, 1932). Each map is converted to percent.

Column 4 gives the length in terms of the leptotene map as described in detail in the text.

Column 5 gives the coincidence as computed by WEINSTEIN's method, eliminating triples involving intermediate loci.

Column 6 gives fraction realized computed as described in the text.

Column 7 gives the product of the total genetic length of the first region, represented by a, and the length of the second region, b for the given experiment. For class 1,4 $a=1=6.8$, $b=4=15.8$, and $1\times4=1.07\times10^{-2}$.

Column 8 lists the total double crossover classes taken directly from the original data. For 1,4 they are 1,4–32; 1,4,6–2; total, $1,4=34$.

Column 9 is obtained by dividing each number in column 8 by 410, which is the total number of double crossovers in column 8. For 1,4, $34/410=8.3\times10^{-2}$.

Column 10 is a summation of column 9 in order of size from column 1. For 6,7, it is equal to $2,3+3,4+6,7$ or in numerical figures, $0.5+2.0+0.7 = 3.2$.

Column 11 is column 9 divided by column 7 and converted to 100 units.

Column 12 is column 11 divided by column 6 and converted to 100 units.

Column 13 is summation of column 11 in order of size.

Column 14 is summation of column 12 in order of size.

Column 15 is column 9 divided by column 6 and converted to percent.

* * * * * *

The tables and figures referred to in the text were drawn up as follows:

Table 1. The locus of any point in this table is the fraction of all recorded genes in that chromosome that lie to the left of it.

In figure 1, the corresponding loci on the leptotene and genetic maps are connected. The total length of each map has been made equal to 100 units.

Figures 2, 5, 6 and 15 have as their co-ordinates columns 6 and 4. In table 6, column 2 is from table 1, and columns 3, 4 and 5 are interpolated from graphs like figure 2.

Figures 3 and 4 have as their co-ordinates columns 6 and 2.

Figures 7 and 9 have as their co-ordinates columns 9 and 4, except for the large dotted circles in figure 7, which have columns 15 and 4.

Figure 8 has as its co-ordinate columns 9 and 2.

Figures 10 and 12 have as their co-ordinates columns 11 and 4.

In figure 11, the upper curves, open circles, have as their co-ordinates columns 14 and 4. The smaller circles are on the curve from which regions having no interference have been omitted. The lower curves, solid circles, have as their co-ordinates columns 13 and 4. The smaller circles here also exclude non-interference regions.

In figure 13, crosses and solid center circles have as their co-ordinates columns 13 and 4, except that classes giving interference are separately summated and drawn. The open circles have as their co-ordinates columns 15 and 4. In this case, only the classes giving interference are drawn and they have been summated in the figure.

Figure 14 corresponds to column 6 of figure 6.

Tables 3a and 4a are reproductions of column 6.

Table 3b is column 9 reproduced for all temperatures.

Table 4b is column 11 reproduced.

Table 4c is taken from the column of classes at the point where column 6 reaches 1.0.

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